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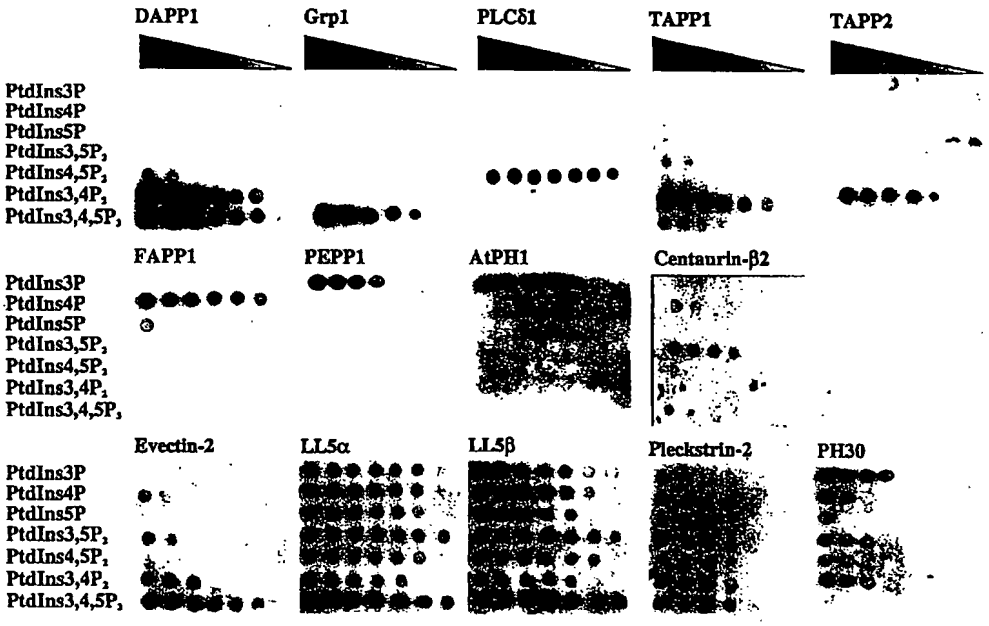

(57) Abstract: The use of a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, in a screening method for identifying a compound suitable for modulating signalling by PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2. The polypeptide preferably comprises a PH domain which binds specifically to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2. The PH domain preferably has at least five of the six residues of a PutativePtdIns(3,4,5)P_3 Binding Motif (PPBM).

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POLYPEPTIDES

The present invention relates to polypeptides, polynucleotides and uses thereof, in particular to polypeptides comprising a PH (pleckstrin homology) domain.

Stimulation of cells with growth factors and insulin activates members of the phosphoinositide 3-kinase (PI 3-kinase) family which phosphorylate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) at the D-3 position of the inositol ring to generate the lipid second messenger, PtdIns(3,4,5)P_3 [1]. A group of proteins has been identified that possess a certain type of pleckstrin homology (PH) domain which interacts specifically with PtdIns(3,4,5)P_3 and often its immediate breakdown product, PtdIns(3,4)P_2, also thought to be a signalling lipid (reviewed in Lemmon & Fergusson (2000) *Biochem J* **350**, 1-18). These include the serine/threonine-specific protein kinases, PKB and PDK1 [2], Bruton's tyrosine kinase BTK [3], the adaptor proteins DAPP1 [4, 5] and Gab1 [6], as well as the ADP Ribosylation Factor (ARF) GTPase activating protein (GAP) centaurin-α [7] and the ARF guanine nucleotide exchange factor, Grp1 [8, 9].

The molecular basis by which certain PH domains are able to interact with PtdIns(3,4,5)P_3 has not been established definitively. However, recent work indicates that six conserved residues that lie at the N-terminal region of the PH domain in a K-X-Sm-X_{6-11}-R/K-X-R-Hyd-Hyd motif (where X is any amino acid, Sm is a small amino acid and Hyd is a hydrophobic amino acid), appear to correlate with high affinity binding of PtdIns(3,4,5)P_3 [10].

To date, all of the specific PtdIns(3,4,5)P_3 binding proteins identified possess this Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM) (Table 1).

TABLE 1: Alignment of proteins possessing a putative PtdIns(3,4,5)P_3 binding motif. Alignment of the amino acid residues of the N-terminal regions of PH domains containing at least five out of the six conserved residues in the motif K-X-Sm-X_{6-11}-R/K-X-R-Hyd-Hyd motif where X is any amino acid, Sm is a small uncharged amino acid and Hyd is a hydrophobic amino acid. * Indicates accession numbers of the EST used to amplify by PCR the PH domain whose lipid binding properties are characterised in Fig 2. Abbreviations used, Org., organisation.

A. Known PtdIns(3,4,5)P_3 binding proteins	Protein	Accession	Species	protein function	Ref.
KEGWLHKRGEYIKY-----WRPRYFLLKND	PKB	S33364	mouse	ser/thr kinase	[2]
ENNLILKMGPVDKRKGLF-ARRRQLLTEGP	PDK1	AAC51825	human	ser/thr kinase	[2]
LESIFLKRSQQKKKTSPLNFKKRLFLLTVH	BTK	Q06187	human	tyrosine kinase	[3]
KEGYLTKQGGLVKT-----WKTRWFTLHRN	DAPP1	AF163254	human	unknown (adaptor?)	[4]
CSGWLRKSPPEKKLKRYA-WKRRWFVLRSG	Gab1	AAC50380	human	docking protein	[6]
KEGYMEKTGPKQTEG----FRKRWFTMDDR	Centaurin-α1	JC7091	human	ARF GAP	[7]
REGWLLKLGGRVKT-----WKRRWFILTDN	GRP1	AF001871	mouse	ARF GEF	[8]
B. PH domains characterised in this study					
KAGYCVKQGAVMKN-----WKRRYFQLDEN	TAPP1	AI216176*	human	unknown (adaptor?)	
KSGYCVKQGNVRKS-----WKRRFFALDDF	TAPP2	AA111410*	mouse	unknown (adaptor?)	
MEGVLYKWTNYLTG-----WQPRWFVLDNG	FAPP1	W32183*	human	unknown (adaptor?)	
IRGWLHKQDSSGLRL----WKRRWFVLSGH	PEPP1	N31123*	human	unknown	
RSGWLTKQGDYIKT-----WRRRWFVLKRG	AtPH1	T04439*	arabidopsis	unknown (adaptor?)	[24]
MEGYLFKRASNAFKT----WNRRWFSIQNS	Centaurin-β2	AA967911*	mouse	ARF GAP	
KSGWLLRQSTILKR-----WKKNWFDLWSD	Evectin-2	AA101447*	human	golgi trafficking?	[21]
CRGYLVKMCGKIKS-----WKKRWFVFDRL	LL5α	AA863428*	human	unknown	[17]
CRGFLIKMCGKIKT-----WKKRWFVFDRN	LL5β	AA461369*	human	unknown	
KEGFLVKRGHIVHN-----WKARWFILRQN	Pleckstrin-2	AI385784*	mouse	cytoskeletal Org.?	[18]
FEGTLYKRGALLKG-----WKPRWFVLNVT	PH30	AI827615*	human	nuclear phosphatase?	[20]

Mutation of certain of the conserved residues in the PPBM in some PH domains has been shown to abolish interaction with PtdIns(3,4,5)P_3 [10]. Significantly, recent structural studies of the PH domain of BTK bound to the head group of PtdIns(3,4,5)P_3 indicate that the basic amino acids in the PPBM may form direct interactions with the monoester phosphate groups of PtdIns(3,4,5)P_3 [11, 12].

We have identified and characterised proteins that bind specifically to a phosphoinositide other than PtdIns(3,4,5)P_3, in particular PtdIns3P, PtdIns3,4P_2 or PtdIns4P. The proteins each possess a PH domain which is considered to contain a PPBM and which binds the said phosphoinositide but not to PtdIns(3,4,5)P_3. These proteins may play important roles in triggering cellular processes that are regulated by other phosphoinositides. The proteins/PH domains may be useful in drug screening assays, in particular for compounds that may be useful in treating cancer, diabetes or stroke. They may also be useful in measuring concentrations and/or locations of the phosphoinositide lipids PtdIns3P, PtdIns3,4P_2 and PtdIns4P.

A first aspect of the invention provides the use of a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, in a screening method for identifying a compound suitable for modulating signalling by PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2.

Polypeptides capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3 have not previously been identified as such. Screening methods making use of such a polypeptide have not previously been proposed.

It is preferred that the polypeptide comprises a PH domain and that the PH domain is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3. It is further preferred that the said PH domain has at least five of the six specified residues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM), or is a variant of such a PH domain that retains the ability to bind to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3.

The term Plecktrin Homology (PH) domain is well known to those skilled in the art. These domains of ~100 residues are found in over 70 other proteins and are predicted to fold into a similar 3-dimensional structures and may mediate protein-lipid, protein-protein interactions, or both (Gibson, T.J. *et al* (1994) *Trends Biochem. Sci.* **19**, 349-353; Shaw, G. (1996) *Bioessays* **18**, 35-46). Polypeptides with PH domains of determined tertiary sructure include plecktrin, spectrin, dynamin, and phospholipase C-γ. Although the percentage identity is poor between PH domains in general there are certain positions that show high levels of residue type conservation. The residues thought to be required for high affinity interaction with PtdIns(3,4,5)P_3 lie in the Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM) near the N-terminal end of the PH domain. A single position (Tryptophan, position 280 of TAPP1 – see Figure 3), near the C-terminal end of the PH domain, shows complete identity throughout the domain family, as shown in Figure 7. Secondary structure predictions indicate that residues 450-530 of PDK1, for example, (positions 1-80) are likely to contain regions of β-sheet, while the residues between 531-550 (positions 80-100) are likely to form an extended α-helix, a prediction that is consistent with the known structures of other PH domains (Gibson, T.J. *et al* (1994) *Trends Biochem. Sci.* **19**, 349-353; Shaw, G. (1996) *Bioessays* **18**, 35-46; [24]).

The term Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM) is also known to those skilled in the art, as discussed above. The motif is K-X-Sm-X_{6-11}-R/K-X-R-Hyd-Hyd motif (where X is any amino acid, Sm is a small, preferably uncharged, amino acid and Hyd is a hydrophobic amino acid) and lies near the N-terminal end of the PH domain. By a small amino acid is included glycine, alanine, threonine and serine. An aspartate or proline amino acid residue (for example) may alternatively be present at the position in the motif where a small amino acid is preferred. By a hydrophobic amino acid is meant tyrosine, leucine, isoleucine, tryptophan and phenylalanine. A glutamine amino acid residue (for example) may alternatively be present at the first position where a hydrophobic amino acid residue is preferred. A glutamine, asparagine or histidine amino acid residue may be present at a position where a lysine or arginine residue is preferred. It is strongly preferred that an acidic or hydrophobic residue is not present at a position where a lysine or arginine residue is preferred, or at the position in the motif where a small amino acid is preferred. It is preferred that the PH domain has at least five of the six specified residues of the PPBM. It is particularly preferred that the PH domain has both hydrophobic amino acids of the motif and/or the first lysine (K) residue of the motif. It is preferred that the PH domain also has a tryptophan residue at the position equivalent to position 280 of TAPP1, as discussed above.

It is preferred that the said polypeptide binds specifically to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 ie is able to bind to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 and is substantially unable to bind to other phosphoinositides, in particular PtdIns5P, PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and three of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and PtdIns(3,5)P_2.

By "able to bind" is meant that binding of the said polypeptide to the said phosphoinositide can be detected using a surface plasmon resonance or protein lipid overlay technique as described in Example 1 and the legends to Table 2 and Figure 4. By "substantially unable to bind" is meant that binding of the said polypeptide to the said phosphoinositide is not detected, or is only weakly detected using a surface plasmon resonance or protein lipid overlay technique as described in Example 1 and the legends to Table 2 and Figure 4. It is preferred that the polypeptide binds to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 with at least two, preferably 3, 5, 10, 15, 20, 30 or 50-fold higher affinity than to other phosphoinositides, in particular PtdIns5P, PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and three of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and PtdIns(3,5)P_2.

It is preferred that the binding of the said polypeptide to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 has an apparent K_D of less than about 2000 nM, 1000 nM or 500 nM, preferably less than about 400 or 350 nM, for example between about 350 nM and 10 nM, when measured using the method described in Example 1. It is preferred that the binding of the said polypeptide to other phosphoinositides, particularly PtdIns5P, PtdIns(4,5)P_2, PtdIns(3,4,5)P_3 and three of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and PtdIns(3,5)P_2, has an apparent K_D of more than about 2000 nM, 1000 nM or 500 nM when measured using the method described in Example 1.

Examples of polypeptides that bind specifically to PtdIns(3,4)P_2 are considered to include mammalian (for example human and mouse) TAPP (for example TAPP1 and TAPP2), and fragments and fusions thereof that comprise the C-terminal PH domain, as discussed further below and in Example 1. Further examples are considered to include fragments, variants,

Table 2: Apparent K_d of PEPP1, FAPP1 wild type and mutant TAPP1 and TAPP2 for binding to phosphoinositides as measured by surface plasmon resonance. The binding of the indicated GST-fusion proteins phosphoinositides incorporated into supported phosphatidylcholine monolayers was measured as described in the experimental section. The affinities (apparent K_d) were determined by global fitting of the association and dissociation curves to a 1:1 binding model. Abbreviations used, FL full length protein; NT-PH, N-terminal PH domain; CT-PH, C-terminal PH domain; NB, no binding detected; ND, not determined.

Phosphoinositide	PEPP1	FAPP1	FL-TAPP1	FL-TAPP2	FL-TAPP1 [R212L]	FL-TAPP1 [R28L]	CT-PH TAPP1	CT-PH TAPP1 [R212L]	NT-PH TAPP1
PtdIns 3*P*	325 nM	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns 4*P*	NB	20 nM	NB	NB	ND	ND	ND	ND	ND
PtdIns 5*P*	NB	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns(3,4)P_2	NB	NB	5 nM	30 nM	NB	28nM	27 nM	NB	NB
PtdIns(3,5)P_2	NB	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns(4,5)P_2	NB	NB	NB	NB	ND	ND	ND	ND	ND

Table 2: Relative affinities of PEPP1, FAPP1 wild type and mutant TAPP1 and TAPP2 for binding to phosphoinositides as measured by surface plasmon resonance. The binding of the indicated GST-fusion proteins phosphoinositides incorporated into supported phosphatidylcholine monolayers was measured as described in the experimental section. The apparent affinities were determined by global fitting of the association and dissociation curves to a 1:1 binding modeland were used to rank the binding affinity relative to that of TAPP1 to PtdIns(3,4)P_2 which was approximately 5 nM. Abbreviations used, FL full length protein; NT-PH, N-terminal PH domain; CT-PH, C-terminal PH domain; NB, no binding detected; ND, not determined.

Phosphoinositide	DAPP1	PDK1	PEPP1	FAPP1	FL-TAPP1	FL-TAPP2	FL-TAPP1 [R212L]	FL-TAPP1 [R28L]	CT-PH TAPP1	CT-PH TAPP1 [R212L]	NT-PH TAPP1
PtdIns 3*P*	NB	NB	65	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns 4*P*	NB	NB	NB	4	NB	NB	ND	ND	ND	ND	ND
PtdIns 5*P*	NB	NB	NB	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns(3,4)P_2	ND	ND	NB	NB	1	5	NB	5.6	5.4	19.6	NB
PtdIns(3,4,5)P_3	0.6	12	NB	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns(3,5)P_2	NB	NB	NB	NB	NB	NB	ND	ND	ND	ND	ND
PtdIns(4,5)P_2	NB	NB	NB	NB	NB	NB	ND	ND	ND	ND	ND

derivatives or fusions thereof, or fusions of fragments, variants or derivatives, that retain the said phosphoinositide binding properties, as discussed further below.

Examples of polypeptides that bind specifically to PtdIns4P are considered to include FAPP, for example mammalian FAPP (for example human or mouse FAPP) or *Xenopus* or Zebrafish FAPP, for example human FAPP1 or FAPP2 and fragments and fusions thereof that comprise a PH domain, as discussed further below and in Example 1. Further examples are considered to include fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or derivatives, that retain the said phosphoinositide binding properties, as discussed further below.

Examples of polypeptides that bind specifically to PtdIns3P are considered to include mammalian (for example human and mouse) PEPP (for example PEPP1, PEPP2 and PEPP3) and plant (for example *Arabidopsis*) AtPH1, and fragments and fusions thereof that comprise a PH domain, as discussed further below and in Example 1. Further examples are considered to include fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or derivatives, that retain the said phosphoinositide binding properties, as discussed further below.

Examples of polypeptides that bind specifically to PtdIns(3,5)P_2 are considered to include centaurin-β2 (for example mammalian, for example human or mouse, or *Drosophila* or *C. elegans*), and fragments and fusions thereof that comprise the C-terminal PH domain, as discussed further below and in Example 1. Further examples are considered to include fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or

derivatives, that retain the said phosphoinositide binding properties, as discussed further below.

Ppreferred fragments of TAPP, PEPP, FAPP, AtPH1 and centaurin-β2 (for example fragments comprising PH domains) are discussed in Example 1, for example in the section relation to cloning of PH domains and in Figure 1.

Suitably, the method comprises the steps of (1) exposing the said polypeptide to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2, in the presence of a test compound; (2) determining whether the test compound modulates binding of the said phosphoinositide to the said polypeptide; and (3) selecting a compound which modulates binding of the said phosphoinositide to the said polypeptide.

Further suitable methods are described in relation to the following aspects of the invention.

A further aspect of the invention provides a method of identifying a compound that modulates the phospholipid binding activity of a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, the method comprising contacting a compound with the said polypeptide or a suitable variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative thereof and determining whether the phospholipid binding activity of the said polypeptide or said variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative thereof is changed in the presence of the compound from that in the absence of said compound. It will be appreciated that the said suitable variant, fragment, derivative or fusion is

capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3.

Preferences and examples are as indicated in relation to the first aspect of the invention.

The binding of polypeptides comprising a PH domain having the required properties to phospholipids is described in Example 1. It is preferred that modulation of the binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 is measured. Methods of detecting binding of the said polypeptide or suitable fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative to phospholipids are described in Example 1 and include a protein-lipid overlay assay in which the lipid is spotted onto a support, for example Hybond-C extra membrane, and protein bound to the support by virtue of interaction with the lipid is detected, for example using an antibody-based method, as well know to those skilled in the art. A surface plasmon resonance assay, for example as described in Example 1 or in Plant *et al* (1995) *Analyt Biochem* **226(2)**, 342-348, may alternatively be used. Methods may make use of a said polypeptide, for example comprising a PH domain, or fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative that is labelled, for example with a radioactive or fluorescent label. Suitable methods may also be described in, for example, Shirai *et al* (1998) *Biochim Biophys Acta* **1402(3)**, 292-302 (use of an affinity column prepared using phosphatidylinositol analogues) and Rao *et al* (1999) *J Biol Chem* **274**, 37893-37900 (use of avidin-coated beads bound to biotinylated phosphatidylinositol analogues).

A further aspect of the invention provides a method of identifying a compound capable of disrupting or preventing the interaction between a polypeptide that is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, and a polypeptide that is capable of binding to the said phosphoinositde-binding polypeptide (interacting polypeptide) wherein the said phosphoinositide-binding polypeptide or a suitable variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative thereof, and/or the interacting polypeptide are exposed to the said compound and the interaction between the phosphoinositide-binding polypeptide or variant, fragment, derivative or fusion and the interacting polypeptide in the presence and absence of the compound is measured.

A further aspect of the invention provides a method of identifying a compound that is capable of binding to a polypeptide that is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3 (interacting polypeptide), wherein the said polypeptide or suitable fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative is exposed to the compound and any binding of the compound to the said polypeptide or fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative is detected and/or measured. The ability of the compound to bind to the said interacting polypeptide may be measured by measuring the ability of the compound to disrupt or prevent the interaction between the phosphoinositide-binding polypeptide (or variant, fragment, derivative or fusion) and the interacting polypeptide.

The binding constant for the binding of the compound to the relevant polypeptide may be determined. Suitable methods for detecting and/or measuring (quantifying) the binding of a compound to a polypeptide are well known to those skilled in the art and may be performed, for example using a method capable of high throughput operation, for example a chip-based method in which the compounds to be tested are immobilised in a microarray on a solid support, as known to those skilled in the art. It is preferred that the said suitable variant, fragment, derivative or fusion of the phosphoinositide binding polypeptide is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3.

In addition, it is preferred that a variant, fragment, derivative or fusion of TAPP comprises the N-terminal of the two PH domains of TAPP. This PH domain may be capable of interacting with polypeptides, as discussed further below. Alternatively (or in addition), it is preferred that a variant, fragment, derivative or fusion of TAPP comprises (preferably as the C-terminal three residues) the last three residues of TAPP (for example TAPP1 or TAPP2), which conform to the minimal sequence motif (Ser/Thr-Xaa-Val/Ile) required for binding to a PDZ domain (as discussed in Example 1); and/or one or more proline rich regions found towards the C-terminus of TAPP2 (as shown in Figure 3 and discussed in Example 1, which may form a binding site for an SH3 domain).

In addition, it is preferred that a variant, fragment, derivative or fusion of FAPP comprises a proline-rich region found toward the C-terminus of FAPP1, which may mediate binding to a SH3 domain (see Figure 5 and Example 1). Similarly, it is preferred that a variant, fragment, derivative or fusion of PEPP comprises one or more proline-rich regions found toward

the C-terminus of PEPP1, which may mediate binding to a SH3 domain (see Figure 6 and Example 1).

It will be understood that it will be desirable to identify compounds that may modulate the activity of the polypeptide *in vivo*. Thus it will be understood that reagents (including any fragment, derivative, variant or fusion of the polypeptide or fusion of a variant, fragment or derivative) and conditions used in the method may be chosen such that the interactions between the said polypeptide and a phosphoinositide, for example PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2, or an interacting polypeptide are substantially the same as between the wild-type, preferably human polypeptide (for example TAPP, PEPP or FAPP) and the phosphoinositide or interacting polypeptide *in vivo*.

A polypeptide that interacts with TAPP, for example TAPP1 or TAPP2 may comprise a PDZ domain and/or a SH3 domain (for TAPP2).

A polypeptide that interacts with PEPP or FAPP may comprise a SH3 domain.

In one embodiment, the compound decreases the relevant binding activity of said polypeptide. For example, the compound may bind substantially reversibly or substantially irreversibly to the relevant binding site of said polypeptide. In a further example, the compound may bind to a portion of said polypeptide that is not the binding site so as to interfere with the binding of the said phosphoinositide-binding polypeptide to the phosphoinositide or interacting polypeptide. In a still further example, the compound may bind to a portion of said polypeptide so as to decrease said

polypeptide's binding activity by an allosteric effect. This allosteric effect may be an allosteric effect that is involved in the natural regulation of the said polypeptide's activity.

The compound may, for example, change the configuration of the polypeptide so that it is substantially unable to bind to the particular phosphoinositide or an interacting polypeptide. The compound may be capable of affecting the intracellular location of the polypeptide; for example, it may inhibit or promote the translocation of the polypeptide to a membrane, for example the plasma membrane or golgi, vacuole, lysosome or endosome membrane. Possible association with cellular membranes of polypeptides comprising a PH domain with the required phosphoinositide binding properties are discussed further in Example 1. The compound may modulate any interaction of the polypeptide with further identical polypeptide molecules (ie self-association, for example dimerisation). It will be appreciated that a compound that, for example, is capable of modulating the phosphorylation or other post-translational modification of the polypeptide may thereby, for example, modulate the ability of the polypeptide to bind to a phosphoinositide or interacting protein. A compound that is capable of modulating the ability of the polypeptide to bind to a phosphoinositide may thereby modulate the intracellular location of the polypeptide molecule and/or modulate any post-translational modification, for example phosphorylation, of the polypeptide.

In a further embodiment, the compound increases the binding activity of said polypeptide. For example, the compound may bind to a portion of said polypeptide that is not the relevant binding site so as to aid the binding of the said polypeptide to the phospholipid or interacting protein, as appropriate. In a still further example, the compound may bind to a portion

of said polypeptide so as to increase said polypeptide's binding activity by an allosteric effect. This allosteric effect may be an allosteric effect that is involved in the natural regulation of the said polypeptide's activity.

An example of a compound that may be capable of inhibiting binding of a phosphoinositide to a said polypeptide is $InsP_4$, the head group of $PtdIns(3,4,5)P_3$. $Ins(1,3,4)P_3$, the head group of $PtdIns(1,3,4)P_3$, may be capable of inhibiting binding of $PtdIns(3,4)P_2$ to TAPP. $Ins(1,3)P_2$, the head group of PtdIns3P, may be capable of inhibiting binding of PtdIns3P to PEPP or AtPH1. $Ins(1,4)P_2$, the head group of PtdIns4P, may be capable of inhibiting binding of PtdIns4P to FAPP. $Ins(1,3,5)P_3$, the head group of $PtdIns(3,5)P_2$, may be capable of inhibiting binding of $PtdIns(3,5)P_2$ to centaurin-β2. A polypeptide comprising an amino acid sequence (preferably C-terminal amino acid sequence) corresponding to the consensus sequence Ser/Thr-Xaa-Val/Ile, for example SDV, may be capable of inhibting binding of TAPP, for example TAPP1 or TAPP2 to an interacting polypeptide comprising a PDZ domain.

Conveniently, the appropriate methods make use of the methods described in Example 1 for detecting and/or quantifying the interaction between a polypeptide and a phospholipid, for example a protein-lipid overlay or surface plasmon resonance method, as discussed above. It is preferred that a GST-tagged fusion of the polypeptide of the invention or a fragment therof is used. Methods in which radioactively or fluorescently labelled lipids are used may also be useful.

Methods of detecting protein-protein interactions are well known to those skilled in the art. The interaction between the said polypeptide or fragment, variant, fusion or derivative thereof or fusion of a fragment, variant or

derivative and an interacting polypeptide may be measured by any method of detecting/measuring a protein/protein interaction, as discussed further below. Suitable methods include yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation methods and cellular response assays. Cellular response assays may be carried out in a variety of cell types, for example in adipocytes or adipocyte cell lines, in a skeletal muscle cell line (such as the L6 myotubule cell line), liver cells or liver cell lines or cancer cells or cancer cell lines.

Skin cancer cells, for example melanoma cells or cell lines, may be particularly preferred when the polypeptide is PEPP or a fragment, variant, fusion or derivative thereof or fusion of a fragment, variant or derivative. Platelets may be preferred when the polypeptide is TAPP. NIH Swiss mouse embryo cells NIH/3T3 (available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (ATCC) as CRL 1658) and human embryonic kidney 293 cells (also available from the ATCC) are examples of cell lines that may be used when investigating the effect of hydrogen peroxide or other cellular stress treatment?

The method may be performed *in vitro*, either in intact cells or tissues, with broken cell or tissue preparations or at least partially purified components. Alternatively, they may be performed *in vivo*. The cells tissues or organisms in/on which the method is performed may be transgenic. In particular they may be transgenic for the said polypeptide capable of binding a specific phosphoinositide.

Preferences for the polypeptide or variant, fragment, fusion or derivative thereof or fusion of a variant, fragment or derivative are as given above.

Other methods of detecting polypeptide/polypeptide interactions include ultrafiltration with ion spray mass spectroscopy/HPLC methods or other physical and analytical methods. Fluorescence Energy Resonance Transfer (FRET) methods, for example, well known to those skilled in the art, may be used, in which binding of two fluorescent labelled entities may be measured by measuring the interaction of the fluorescent labels when in close proximity to each other.

This may be done in a whole cell system or using purified or partially purified components. Similarly, expression of a protein encoded by an RNA transcribed from a promoter regulated by the polypeptide may be measured. The protein may be one that is physiologically regulated by the polypeptide or may be a "reporter" protein, as well known to those skilled in the art (ie a recombinant construct may be used). A reporter protein may be one whose activity may easily be assayed, for example (β-galactosidase, chloramphenicol acetyltransferase or luciferase (see, for example, Tan *et al*(1996)). In a further example, the reporter gene may be fatal to the cells, or alternatively may allow cells to survive under otherwise fatal conditions. Cell survival can then be measured, for example using colorimetric assays for mitochondrial activity, such as reduction of WST-1 (Boehringer). WST-1 is a formosan dye that undergoes a change in absorbance on receiving electrons via succinate dehydrogenase.

Promoters whose activity may be regulated by a signalling pathway in which the polypeptide may be involved may be identified using microarray technology, as known to those skilled in the art, in which the expression of multiple genes may be examined simultaneously, for example in stimulated and unstimulated cells expressing the wild-type polypeptide or a dominant negative mutation. Differences in expression patterns between the different

cells/activation states indicate genes/promoters which the polypeptide may regulate. An example of a dominant negative mutant of TAPP is a fragment of TAPP comrpising the C-terminal PH domain, but not the N-terminal PH domain and/or putative SH3 binding domain (TAPP2) and/or PDZ binding sequence. Thus, transcription of these genes may be assessed or the promoter for such a gene may be used in a reporter construct as described above.

Insulin exerts important effects on gene expression in multiple tissues (O'Brien, R. M. & Granner, D. K (1996) *Physiol. Rev.* **76**, 1109-1161). In the liver, insulin suppresses the expression of a number of genes which contain a conserved insulin response sequence (IRS)[1] (CAAAAC/TAA), including insulin-like growth factor binding protein-1 (IGFBP-1), apolipoprotein CIII (apoCIII), phosphoenol-pyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase) (Goswami, R *et al* (1994) *Endocrinol.* **134,** 2531-2539; Suwanickul, A *et al* (1993) *J. Biol Chem.* **268,** 17063-17068; Li, W. W *et al* (1995) *J. Clin. Invest* **96,** 2601-2605; O'Brien, R. M *et al* (1990) *Science* **249,** 533-537; Streeper, R. S *et al* (1997) *J. Biol Chem.* **272,** 11698-11701). Thus, transcription of these genes may be assessed or promoters from these genes may be used in a reporter construct as described above, for example when the polypeptide is TAPP. Microarray technology may be used in assessing transcription of genes or reporter constructs, as known to those skilled in the art.

The transcription of a gene indicated above (or any other that is regulated by cellular stress, a growth factor or insulin signalling) may be measured by measurement of changes in the enzymatic or other activity of the said gene product, for example in a cell. Suitable methods will be well known to those skilled in the art.

It will be necessary to perform various control assays, as known to those skilled in the art, in order to determine that a compound is affecting signalling *via* the said phosphoinositide-binding polypeptide, rather than having some other effect on processes leading to whatever measurement is made. For example, it may be necessary to determine what effect the compound being tested has on the activity rather than the activation of a polypeptide, for example a protein kinase, that may be acting downstream (in the signalling pathway) of the said phosphoinositide-binding polypeptide but upstream of the effect being measured.

A further aspect of the invention provides a method of identifying a polypeptide (interacting polypeptide) that interacts with a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, the method comprising 1) contacting a) the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative with b) a composition that may contain such an interacting polypeptide, 2) detecting the presence of a complex containing the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative and an interacting polypeptide, and optionally 3) identifying any interacting polypeptide bound to the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative.

Preferences in relation to the said suitable fragment, variant, derivative or fusion include those indicated above in relation to the previous aspect of the

invention. It will be appreciated that the method may be carried out in a cell, for example a recombinant cell. The cell may be recombinant in relation to the said phosphoinositide-binding polypeptide and/or in relation to a putative interacting polypeptide or a polypeptide thought to be involved in signalling *via* the said phosphoinositide-binding polypeptide, for example a polypeptide involved in platelet activation, for example integrin receptors.

The interaction between the phosphoinositide-binding polypeptide or fragment, variant, deriviative or fusion and the interacting polypeptide may be measured by any method of detecting/measuring a protein/protein interaction, as discussed further below. Suitable methods include yeast two-hybrid interactions, co-purification, ELISA, co-immunoprecipitation methods and cellular response assays. Cellular response assays may be carried out in a suitable cell or cell line as discussed above, for example in adipocytes or adipocyte cell lines, hepatocyte cells or cell lines, myotube cells or cell lines, cancer cells or cell lines, particularly melanoma cells, for example the G361 melanoma cell line, as discussed in Example 1, or in platelets. Heart, skeletal muscle, kidney or placenta cells or cell lines (or other tissue types indicated in Table 3 as a source of TAPP clones) may be particularly suitable in relation to TAPP. Cells or cell lines from tissue types indicated in Table 3 as a source of FAPP or centaurin-β2 clones may be particularly suitable in relation to FAPP or centaurin-β2, respectively. Skin or cancer cells or cell lines, particularly melanoma cell lines (for example the G361 cell line), may be particularly suitable in relation to PEPP.

A further method of identifying the interacting polypeptide of the invention includes expression cloning which makes use of the transfection of cDNAs from a cellular source which is believed to encode the interacting

Protein	Species	Tissue	NCBI Accession (I.M.A.G.E. Clone ID)
TAPP1	Human	Parathyroid tumour	W56032, W63712 (326517)
		Foetal heart	AA054961
		Lung	AI191308, AI216176 (1884429)
		Colon	AI709038
		Kidney	AA875839, AI343801
		Skeletal muscle	AA211648
		Melanocyte	N31136
		Testes	AI343801
		Olfactory epithelium	AL046495
		Germinal centre B cell	AA740729 (1286305)
		Foetal Liver	H78048, H90955
		Uterus	AA150283 (491669)
		Placenta	R62858
		Testis	AA429617
		Foetal liver	R91752
	Mouse	Thymus	AA762924
		Kidney	AI987596 (2158944)
		Embryo	AA388896 (569145)
	Zebrafish	Pooled	AI497344, AI878142
		Fin regenerates	AW595189
TAPP2	Human	Germinal centre B cells	AA721234 (1300983)
		Foetal lung	AI185428 (1742690)
		Pooled tumours	AA975814 (1589519)
		Brain	AA985353, AW408638
	Mouse	Embryo	AA111410 (557355)
		Thymus	AA118260, AI447504 (574391)
		Myotubes	AI592480, AI591454 (1162924)
	Zebrafish	Pooled	AI497344, AI878142
	chicken	Bursa of Fabricius	AJ393764, AJ395418,AJ393899
FAPP1	Human	Multiple sclerosis	N79274 (287618)
		Germinal centre B cells	AA481205 (815143), AA481224 (815169), AI221252 (1842552)
		Bowel	BE136879
		Testis	AA431220
		Lung carcinoid	AW340998, AW341035
		Foetal heart	W73345
		Colon tumour	AI337400
		Pancreatic islet	W52895 (338749)
		Aorta endothelial	AA301959
		Germ cell tumour	AI341371
		Pooled	AI246428, AI242688, AA453702 (813820), AA724575 (1327281)
		Parathyroid tumour	
	Mouse	Uterus	W32183 (321321)
		Total fetus	AI161122 (1721404)
		Embryo	AA463817 (796517)
		Macrophages	AA681116 (1134498)
		Tumour	AA867335 (1293870)
		Spleen	AW412246 (2812588)
	Rat	Total foetus	AA184412
		Heart	AA048334 (477463)
	Xenopus	Ovary	AA419963 (847595)
	Zebrafish	Pooled	AI177017
		Unfertilised egg	AI071963

		Pooled	AW644282 AW174299
PEPP1	Human	Melanocytes Melanoma	N49341 (272085), N31123 (265349) AL135424 (DKFZp762M2115), AL135565
PEPP2	Human	Kidney Brain Foetal liver and spleen Germ cell line	A1808805 AA232124 W91917 AI638629

Table 3. Tissue origin of ESTs encoding TAPP1, TAPP2, PEPP1, and FAPP1. ESTs which we have sequenced have their I.M.A.G.E. Consortium Clone ID in parentheses.

polypeptide (such as a receptor) into a suitable cell line (such as a CHO cell line or Hep2A3 cell line) such that at least some of the cell lines express the interacting polypeptide. Cell lines expressing the interacting polypeptide are selected based on the ability of a labelled (for example radiolabelled) said phosphoinositide binding polypeptide (or suitable fragment, variant, derivative or fusion thereof, or fusion of a fragment, variant or derivative) to bind to the transfected cell line but not to the non-transfected cell line.

The method may be performed *in vitro*, either in intact cells or tissues, with broken cell or tissue preparations or at least partially purified components. Alternatively, they may be performed *in vivo*. The cells tissues or organisms in/on which the method is performed may be transgenic. In particular they may be transgenic for the said phosphoinositide-binding polypeptide.

Preferences for the phosphoinositide-binding polypeptide or fragment, variant, derivative or fusion thereof, for example a processed polypeptide of the invention are as given above.

A further aspect of the invention provides a substantially pure interacting polypeptide identified or identifiable by the method of the invention described above. A still further aspect of the invention provides a recombinant polynucleotide encoding or suitable for expressing the interacting polypeptide of the invention. A still further aspect of the invention provides a nucleic acid complementary to a nucleic acid encoding or capable of expressing the interacting polypeptide of the invention. Methods of identifying, preparing or isolating the said nucleic acid will be well known to those skilled in the art.

The following methods of isolating a nucleic acid encoding a polypeptide of the invention (for example an interacting polypeptide of the invention or a phosphinositide-binding polypeptide of the invention, as discussed further below) are given for purposes of illustration and are not considered to be exhaustive.

The polypeptide may be cleaved, for example using trypsin, cyanogen bromide, V8 protease formic acid, or another specific cleavage reagent. The digest may be chromatographed on a Vydac C18 column or subjected to SDS-PAGE to resolve the peptides. The N-terminal sequence of the peptides may then be determined using standard methods.

The sequences are used to isolate a nucleic acid encoding the peptide sequences using standard PCR-based strategies. Degenerate oligonucleotide mixtures, each comprising a mixture of all possible sequences encoding a part of the peptide sequences, are designed and used as PCR primers or probes for hybridisation analysis of PCR products after Southern blotting. mRNA prepared from cells in which the polypeptide

may be expressed is used as the template for reverse transcriptase, to prepare cDNA, which is then used as the template for the PCR reactions.

Positive PCR fragments are subcloned and used to screen cDNA libraries to isolate a full length clone for the polypeptide.

Alternatively, the sequences of initial subcloned PCR fragments may be determined, and the sequence may then be extended by known PCR-based techniques to obtain a full length sequence.

Alternatively, the initial PCR sequence may be used to screen electronic databases of expressed sequence tags (ESTs) or other known sequences. By this means, related sequences may be identified which may be useful in isolating a full length sequence using the two approaches described above.

Sequences are determined using the Sanger dideoxy method. The encoded amino acid sequences may be deduced by routine methods.

Techniques used are essentially as described in Sambrook *et al* (1989) Molecular cloning, a laboratory manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Alternatively, antibodies may be raised against the polypeptide.

The antibodies are used to screen a λgt11 expression library made from cDNA copied from mRNA from cells in which the polypeptide may be expressed.

Positive clones are identified and the insert sequenced by the Sanger method as mentioned above. The encoded amino acid sequence may be deduced by routine methods.

It will be appreciated that it may be desirable to express the polypeptide encoded by the isolated nucleic acid in order to determine that the polypeptide has the expected properties, for example expected ability to bind to a said phosphoinositide-binding polypeptide, for example TAPP, PEPP, FAPP, AtPH1 or centaurin-β2.

It will be appreciated that the above methods of the invention may be performed within a cell, for example using the yeast two hybrid system as is well known in the art. It will further be appreciated that a transgenic animal in which a said phosphoinositide-binding polypeptide gene is altered and/or a recombinant said phosphoinositide-binding polypeptide gene is present, for example a rodent, in particular a mouse, may be useful in, for example, identifying polypeptides that interact with the said phosphoinositide-binding polypeptide.

The interacting polypeptide may be a receptor molecule, for example a receptor molecule present in/on the surface of a cell, for example a platelet, adipocyte, muscle or skin cell. The receptor molecule may be a transmembrane polypeptide or complex, as know to those skilled in the art. It will be appreciated that known receptors, for example platelet integrin receptors, are not included.

It will be appreciated that screening assays which are capable of high throughput operation will be particularly preferred. Examples may include cell based assays and protein-protein binding assays. An SPA-based

(Scintillation Proximity Assay; Amersham International) system may be used. For example, beads comprising scintillant and an interacting polypeptide (which term it will be appreciated includes a polypeptide which capable of interacting with a polypeptide of the invention or fragment thereof and is a fragment of a polypeptide, for example a naturally occuring polypeptide, that is also capable of interacting with a polypeptide of the invention or fragment thereof) may be prepared. The beads may be mixed with a sample comprising, for example, the phosphatidylinositol-binding polypeptide into which a radioactive label has been incorporated and with the test compound. Conveniently this is done in a 96-well format. The plate is then counted using a suitable scintillation counter, using known parameters for the particular radioactive label in an SPA assay. Only the radioactive label that is in proximity to the scintillant, ie only that bound to the phosphoinositide-binding polypeptide that is bound to the interacting polypeptide anchored on the beads, is detected. Variants of such an assay, for example in which the interacting polypeptide is immobilised on the scintillant beads *via* binding to an antibody or antibody fragment, may also be used. Phosphoinositides or analogues thereof may be immobilised on SPA beads, for example using methods as described in Shirai *et al* (1998) *Biochim Biophys Acta* **1402(3)**, 292-302 or in Rao *et al* (1999) *J Biol Chem* **274**, 37893-37900.

It will be appreciated that the screening assays of the invention are useful for identifying compounds which may be useful in the treatment of diabetes, defects of glycogen metabolism, cancer (including melanoma), inflammatory conditions, ischaemic conditions, for example stroke, thrombosis or tendency to thrombosis (for example useful as an antithrombotic agent).

The compound may be a drug-like compound or lead compound for the development of a drug-like compound for each of the above methods of identifying a compound. It will be appreciated that the said methods may be useful as screening assays in the development of pharmaceutical compounds or drugs, as well known to those skilled in the art.

The term "drug-like compound" is well known to those skilled in the art, and may include the meaning of a compound that has characteristics that may make it suitable for use in medicine, for example as the active ingredient in a medicament. Thus, for example, a drug-like compound may be a molecule that may be synthesised by the techniques of organic chemistry, less preferably by techniques of molecular biology or biochemistry, and is preferably a small molecule, which may be of less than 5000 daltons molecular weight. A drug-like compound may additionally exhibit features of selective interaction with a particular protein or proteins and be bioavailable and/or able to penetrate cellular membranes, but it will be appreciated that these features are not essential.

The term "lead compound" is similarly well known to those skilled in the art, and may include the meaning that the compound, whilst not itself suitable for use as a drug (for example because it is only weakly potent against its intended target, non-selective in its action, unstable, difficult to synthesise or has poor bioavailability) may provide a starting-point for the design of other compounds that may have more desirable characteristics.

It will be appreciated that the compound may be a polypeptide that is capable of competing with the polypeptide of the invention for binding to the interacting polypeptide. Thus, it will be appreciated that a screening method as described above may be useful in identifying polypeptides that

may also interact with the interacting polypeptide, for example a receptor molecule.

It will be understood that it will be desirable to identify compounds that may modulate the activity of the polypeptide(s) *in vivo*. Thus it will be understood that reagents and conditions used in the method may be chosen such that the interactions between the said polypeptide and the interacting polypeptide are substantially the same as between the said polypeptide or a fragment therof and a naturally occuring interacting polypeptide *in vivo*.

The "drug-like compounds" and "lead compounds" identified in the screening assays of the invention are suitably screened in further screens to determine their potential usefulness in treating diabetes, defects of glycogen metabolism, cancer (including melanoma), inflammatory conditions, ischaemic conditions, for example stroke, or thrombosis or tendency to thrombosis. Additional screens which may be carried out include determining the effect of the compounds on blood glucose levels, tumour growth or blood clotting tendency/time, as appropriate. This may typically be done in rodents.

A further aspect of the invention is a kit of parts useful in carrying out a method, for example a screening method, of the invention. Such a kit may comprise a said phosphoinositide-binding polypeptide (or a suitable fragment, variant, derivative or fusion thereof, or fusion of a fragment, variant or derivative) and an interacting polypeptide, for example a receptor molecule.

A further aspect of the invention provides a compound identified by or identifiable by the screening method of the invention. The compound may

be an antibody capable of binding to the said phosphoinositide-binding polypeptide or interacting polypeptide, as discussed further below, or it may be a peptide derivable from the said phosphoinositide-binding polypeptide or interacting polypeptide (ie a fragment of the said polypeptide).

It will be appreciated that such a compound may be an inhibitor of the formation or stability of a complex of the phosphoinositide-binding polypeptide of the invention or a variant, fragment, derivative or fusion used in the screen, with an interacting polypeptide(s), for example a receptor, and therefore ultimately a modulator of any activity of that complex, for example any signalling activity, for example protein kinase activity or protein phosphatase activity. The intention of the screen may be to identify compounds that act as modulators, for example inhibitors or promoters, preferably inhibitors of the activity of the complex, even if the screen makes use of a binding assay rather than an activity (for example signalling activity) assay. It will be appreciated that the action of a compound found to bind the interacting polypeptide may be confirmed by performing an assay of, for example, protein kinase activity in the presence of the compound. It will be appreciated that a compound that interacts with an interacting polypeptide that is (or that interacts with) a receptor molecule may act as an agonist or antagonist of any signalling activity of the said receptor.

A further aspect of the invention provides a method of disrupting or preventing the interaction between a said phosphoinositide-binding polypeptide or a variant, fragment, derivative or fusion, or a fusion of a variant, fragment or derivative, and an interacting polypeptide, for example a receptor molecule, as defined above wherein the said interacting polypeptide or phosphoinositide-binding polypeptide of the invention or a

variant, fragment, derivative or fusion, or a fusion of a variant, fragment or derivative is exposed to a compound of the invention (which may be an antibody of the invention, as discussed further below).

Preferences for the phosphoinositide-binding polypeptide and the interacting polypeptide are as set out in relation to earlier aspects of the invention. It is particularly preferred that the phosphoinositide-binding polypeptide (or variant, fragment, derivative or fusion) or interacting polypeptide is a naturally occurring polypeptide or naturally occurring allelic variant thereof.

Conveniently, the said phosphoinositide-binding polypeptide or fragment, derivative, variant or fusion used in the methods is one which is produced by recombinant DNA technology. Similarly, it is preferred if the interacting polypeptide used in the methods, for example of identifying compounds that modulate the interaction with the said phosphoinositide-binding polypeptide, is one which is produced by recombinant DNA technology.

It will be appreciated that it may be desirable to carry out a method of the invention, for example a compound screening method of the invention, in the presence of the phosphoinositide to which the said phosphoinositide-binding protein is capable of binding. Expression of a constitutively active phosphoinositide (PI) kinase may be desirable in relation to a cell-based assay, in order to elevate the level of the appropriate phosphoinositide in the cell. For example, (over)expression of a Class 1A PI3 kinase may be useful in relation to TAPP, as it may increase the level of PtdIns(3,4,5)P_3 and thereby the level of PtdIns(3,4)P_2. Overexpression of a Class II PI3 kinase may be useful in relation to PEPP or AtPH1, as it may increase the level of PtdIns3P, whilst overexpression of a PI4 kinase may be useful in relation to

FAPP, as it may increase the level of PtdIns4P. Overexpression of Fab1[38, 39] may be useful in relation to centaurin-β2, as it may increase the level of $Ptd(3,5)P_2$.

It will be appreciated that by "suitable" we mean that the said components in the method are those that have interactions or activities which are substantially the same as those of the said phosphoinositide-binding polypeptide or an interacting polypeptide or as the case may be but which may be more convenient to use in an assay. For example, fusions of the said phosphoinositide-binding polypeptide are particularly useful since said fusion may contain a moiety which may allow the fusion to be purified readily.

A further aspect of the invention provides a method of detecting and/or quantifying $PtdIns(3,4)P_2$, PtdIns3P, PtdIns4P or $PtdIns(3,5)P_2$ in a sample wherein the sample is exposed to a polypeptide capable of binding to $PtdIns(3,4)P_2$, PtdIns3P, PtdIns4P or $PtdIns(3,5)P_2$ but not capable of binding to $PtdIns(3,4,5)P_3$ and the binding of the said polypeptide to any said phosphoinositide present is detected. Preferences for the said polypeptide are as indicated in relation to the first aspect of the invention. Methods of detecting binding of the said phosphoinositide to the said polypeptide are discussed above and in Examples 1 and 3. The polypeptides may be used to determine the location of the said phosphoinositide using *in situ* techniques, as well known to those skilled in the art. The cells may be living cells, or fixed using conventional methods, for example formaldehyde fixing. Particularly in relation to investigating living cells, it is preferred that the said polypeptide comprises a chromophore, for example a green fluorescent protein moiety (GFP; including mutated GFPs, for example blue, yellow or cyan fluorescent

proteins), for example as a fusion protein which is expressed in the cell, as well known to those skilled in the art. GFPs are produced naturally by *Aequorea victoria* but, as is well known in the art and described, for example, in Mitra *et al* (1996) *Gene* **173**, 13-17; Cubitt *et al* (1995) *Trends Biochem. Sci.* **20**, 448-454; Miyawaki *et al* (1997) *Nature* **388**, 882-887; Patterson *et al* (1997) *Biophys J.* **73**, 2782-2690; Heim & Tsien (1996) *Curr. Biol.* **6**, 178-182; and Heim *et al* (1995) *Nature* **373**, 663-664, mutant GFPs are available which have modified spectral characteristics. Certain GFPs and mutant GFPs are available from Clontech Laboratories UK Ltd, Wade Road, Basingstoke, Hants RG24 8NE.

The methods may be used in assays for detecting or quantifying (measuring) enzyme activity, for example lipid phosphatases or inositol lipid kinases, for example Fab1p (a stress-activated phosphatidylinositol 3-phosphate 5-kinase), which converts PtdIns3P to PtdIns(3,5)P_2. Thus, a PH domain which binds to PtdIns3P (for example the PH domain of PEPP1 or AtPH1) may be used to monitor the level of PtdIns3P and thereby Fab1p activity. This is discussed further in Example 3. Such a lipid kinase/phosphatase assay may be performed *in vitro* (for example using techniques described above and in Examples 1 and 3) or *in vivo*, for example in cells, using techniques as described above. The methods may be used in identifying modulators (for example inhibitors or activators) of the enzyme activity, as will be apparent to those skilled in the art. Thus, the invention provides a method for identifying a modulator of a lipid kinase or phosphatase activity wherein the lipid kinase or phosphatase activity is measured in the presence (and preferably also in the absence, or in the presence of more than one concentration) of the compound using such a method. The invention further provides a kit of parts useful in carrying out such a detection/quantification or screening method. Suitable components

for such a kit include reagents and enzymes of the types mentioned in Example 3, for example a PH domain of the invention and a phosphoinositide which binds to the said PH domain or a lipid which is converted into a phosphoinositide which binds to the said PH domain by an enzyme, for example lipid kinase or phosphatase.

A further aspect of the invention provides a substantially pure polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, wherein the polypeptide is not full length centaurin-β2 or full length AtPH1[19]. Preferably the polypeptide comprises a PH domain. Still more preferably, the PH domain is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and/or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3, and has at least five of the six residues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM). Further preferences for the said phosphoinositide-binding polypeptide of the invention, for example concerning phosphoinositide binding specificity, are as indicated above in relation to the phosphoinositide-binding polypeptide in relation to the screening/use aspects of the invention.

It is not considered that a PI 4-kinase polypeptide (or recombinant polypeptide comprising a PH domain therefrom) as described in Stevenson *et al* (1998) *J Biol Chem* **273**, 22761-22767 is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and/or PtdIns(3,5)P_2) but is not capable of binding to PtdIns(3,4,5)P_3. For the avoidance of doubt, the polypeptides described in Stevenson *et al* (1998) (ie PI 4 kinases and PH domains thereof from *Arabidopsis*, carrot, yeast STT4, rat, human PI4Kα and bovine brain PI4K200 are excluded from the polypeptides of the invention. These polypeptides are further not considered to comprise a PH domain which has

at least five of the six residues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM).

It is not considered that PLCδ_1 is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P and/or PtdIns(3,5)P_2) but is not capable of binding to PtdIns(3,4,5)P_3. For the avoidance of doubt, PLCδ_1 is excluded from the polypeptides of the invention.

A polypeptide of the invention may be useful in accordance with the uses or screens of the preceding aspects of the invention, as indicated above. Examples of polypeptides of the invention include TAPP (for example TAPP1 and TAPP2), PEPP (for example PEPP1, PEPP2 and PEPP3) and FAPP (for example FAPP1 or FAPP2) and fragments, variants, derivatives or fusions thereof, or fusions of fragments, variants or derivatives, for example a fragment comprising a phosphoinositide-binding PH domain. It is preferred that the said fragment, variant, derivative or fusion retains the phosphoinositide binding properties of the polypeptide of the invention from which it is derived/derivable, as discussed further below.

Centaurin-β2 and AtPH1 or fragments, derivatives, variants or fusions either thereof, or fusions of such fragments, derivatives or variants, which retain the said phosphoinositide lipid binding properties may also be useful in accordance with the use and methods of the first aspect of the invention. Suitable fragments are described in Example 1. Typically a suitable fragment will comprise the PH domain (or a variant thereof) of centaurin-β2 or AtPH1. Such fragments or fusions, derivatives or variants thereof (that are not full length AtPH1 or centaurin-β2) are polypeptides of the invention.

A further aspect of the invention provides a substantially pure polypeptide comprising the amino acid sequence

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQNLP
SGSSRVGAIKLTYISKVSDATKLRPKAEFCFVMNAGMRKYFLQANDQQDL
VEWVNVLNKAIKITVPKQSDSQPNSDNLSRHGECGKKQVSYRTDIVGGVP
IITPTQKEEVNECGESIDRNNLKRSQSHLPYFTPKPPQDSAVIKAGYCVK
QGAVMKNWKRRYFQLDENTIGYFKSELEKEPLRVIPLKEVHKVQECKQSD
IMMRDNLFEIVTTSRTFYVQADSPEEMHSWIKAVSGAIVAQRGPGRSASS
EHPPGPSESKHAFRPTNAAAATSHSTASRSNSLVSTFTMEKRGFYESLAK
VKPGNFKVQTVSPREPASKVTEQALLRPQSKNGPQEKDCDLVDLDDASLP
VSDV

(human TAPP1 amino acid sequence; see also Accession No AF286160)

or

RGEREARRVWQADPEIPGARRTRRPEGRPRPM*RAPPEPRPLHGGG*CEQ
SPGMPYVDRQNRICGFLDIEEHENSGKFLRRYFILDTQANCLLWYMDNPQ
NLAMGAGAVGALQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQAND
QKDMKDWVEALNQASKITVPKGGGLPMTTEVLKSLAAPPALEKKPQVAYK
TEIIGGVVVHTPISQNGGDGQEGSEPGSHTILRRSQSYIPTSGCRASTGP
PLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIFFKDV
LKTHECLVKSGDLLMRDNLFEIITSSRTFYVQADSPEDMHSWIKEIGAAV
QALKCHP

(partial human TAPP2 amino acid sequence)

or

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
nMNAGMRKYFLQANDQQDLVEWVNVLNKAIKITVPKQSDSQPASDSLSRQ
GDCGKKQVSYRTDIVGGVPIITPTQKEEVNECGESLDRNNLKRSQSHLPY
FAPKPPSDSAVIKAGYCVKQGAVMKNWKRRYFQLDENTIGYFKSELEKEP
LRVIPLKEVHKVQECKQSDIMMRDNLFEIVTTSRTFYVQADSPEEMHSWI
KAVSGAIVAQRGPGRSSSSnnnnnnnnnnnnnnnnnnnnnnnnnnnnn

(partial mouse TAPP1 amino acid sequence; the run of n's indicates a gap of unknown length)

or

MPYVDRQNRICGFLDIEDNENSGKFLRRYFILDTQANCLLWYMDNPQNLA
VGAGAVGSLQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQANDQKD
LKDWVEALNQASKITVPKAGTVPLATEVLKNLTAPPTLEKKPQVAYKTEI
IGGVVVQTPISQNGGDGQEGCEPGTHAFLRRSQSYIPTSGCRPSTGPPLI
KSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIPLKDVLKT
HECLVKSGDLLMRDNLFEIITTSRTFYVQADSPEDMHSWIEGIGAAVQAL
KCHPREPSFSRSISLTRPGSSTLTSAPNSILSRRRPPAEEKRGLCKAPSV
ASSWQPWTPVPQAEEKPLSVEHAPEDSLFMPNPGESTATGVLASSRVRHR
SEPQHPKEKPFVFNLDDENIRTSDV

(mouse TAPP2 amino acid sequence; see also Accession No AF286161) or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

Further TAPP polypeptides include the chicken TAPP2 sequence as given in Accession No AF302149. Human TAPP2 may have the sequence given in Accession No AF 286164, which is a fragment of the sequence given above, as follows:

MPYVDRQNRICGFLDIEEHENSGKFLRRYFILDTQANCLLWYMDNPQ
NLAMGAGAVGALQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQAND
QKDMKDWVEALNQASKITVPKGGGLPMTTEVLKSLAAPPALEKKPQVAYK
TEIIGGVVVHTPISQNGGDGQEGSEPGSHTILRRSQSYIPTSGCRASTGP
PLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIFFKDV
LKTHECLVKSGDLLMRDNLFEIITSSRTFYVQADSPEDMHSWIKEIGAAV
QALKCHP

A further aspect of the invention provides a substantially pure polypeptide comprising the amino acid sequence

MEGSRPRSSLSLASSASTISSLSSLSPKKPTRAVNKIHAFGKRGNALRRD
PNLPVHIRGWLHKQDSSGLRLWKRRWFVLSGHCLFYYKDSREESVLGSVL
LPSYNIRPDGPGAPRGRRFTFTAEHPGMRTYVLAADTLEDLRGWLRALGR
ASRAEGDDYGQPRSPARPQPGEGPGGPGGPPEVSRGEEGRISESPEVTRL
SRGRGRPRLLTPSPTTDLHSGLQMRRARSPDLFTPLSRPPSPLSLPRPRS

APARRPPAPSGDT

(partial human PEPP1 amino acid sequence)

or

MEGSRPRSSLSLASSASTISSLSSLSPKKPTRAVNKIHAFGKRGNALRRDP
NLPVHIRGWLHKQDSSGLRLWKRRWFVLSGHCLFYYKDSREESVLGSVLLP
SYNIRPDGPGAPRGRRFTFTAEHPGMRTYVLAADTLEDLRGWLRALGRASR
AEGDDYGQPRSPARPQPGEGPGGPGGPPEVSRGEEGRISESPEVTRLSRGR
GRPRLLTPSPTTDLHSGLQMRRARSPDLFTPLSRPPSPLSLPRPRSAPARR
PPAPSGDTAPPARPHTPLSRIDVRPPLDWGPQRQTLSRPPTPRRGPPSEAG
GGKPPRSPQHWSQEPRTQAHSGSPTYLQLPPRPPGTRASMVLLPGPPLEST
FHQSLETDTLLTKLCGQDRLLRRLQEEIDQKQEEKEQLEAALELTRQQLGQ
ATREAGAPGRAWGRQRLLQDRLVSVRATLCHLTQERERVWDTYSGLEQELG
TLRETLEYLLHLGSPQDRVSAQQQLWMVEDTLAGLGGPQKPPPHTEPDSPS
PVLQGEESSERESLPESLELSSPRSPETDWGRPPGGDKDLASPHLGLGSPR
VSRASSPEGRHLPSPQLGTKAPVARPRMNAQEQLERMRRNQECGRPFPRPT
SPRLLTLGRTLSPARRQPDVEQRPVVGHSGAQKWLRSSGSWSSPRNTTPYL
PTSEGHRERVLSLSQALATEASQWHRMMTGGNLDSQGDPLPGVPLPPSDPT
RQETPPPRSPPVANSGSTGFSRRGSGRGGGPTPWGPAWDAGIAPPVLPQDE
GAWPLRVTLLQSSL

(human PEPP1 amino acid sequence; see also Accession No AY007233)

or

CKHPVTGQPSQDNCIFVVNEQTVATMTSEEKKERPISMINEASNYNVTSD
YAVHPMSPVGRTSRASKKVHNFGKRSNSIKRNPNAPVVRRGWLYKQDSTG
MKLWKKRWFVLSDLCLFYYRDEKEEGILGSILLPSFQIALLTSEDHINRK
YAFKAAHPNMRTYYFCTDTGKEMELWMKAMLDAALVQTEPVKRVDKITSE
NAPTKETNNIPNHRVLIKPEIQNNQKNKEMSKIEEKKALEAEKYGFQKDG
QDRPLTKINSVKLNSLPSEYESGSACPAQTVHYRPINLSSSENKIVNVSL
ADLRGGNRPNTGPLYTEADRVIQRTNSMQQLEQWIKIQKGRGHEEETRGV
ISYQTLPRNMPSHRAQIMARYPEGYRTLPRNSKTRPESICSVTPSTHDKT
LGPGAEEKRRSMRDDTMWQLYEWQQRQFYNKQSTLPRHSTLSSPKTMVNI
SDQTMHSIPTSPSHGSIAAYQGYSPQRTYRSEVSSPIQRGDVTIDRRHRA
HHPKVK

(partial human PEPP2 amino acid sequence)

or

MAADLNLEWISLPRSWTYGITRGGRVFFINEEAKSTTWLHPVTGEAVVTG

HRRQSTDLPTGWEEAYTFKGARYYINHNERKVTCKHPVTGQPSQDNCIFV
VNEQTVATMTSEEKKERPISMINEASNYNVTSDYAVHPMSPVGRTSRASK
KVHNFGKRSNSIKRNPNAPVVRRGWLYKQDSTGMKLWKKRWFVLSDLCLF
YYRDEKEEGILGSILLPSFQIALLTSEDHINRKYAFKAAHPNMRTYYFCT
DTGKEMELWMKAMLDAALVQTEPVKRVDKITSENAPTKETNNIPNHRVLI
KPEIQNNQKNKEMSKIEEKKALEAEKYGFQKDGQDRPLTKINSVKLNSLP
SEYESGSACPAQTVHYRPINLSSSENKIVNVSLADLRGGNRPNTGPLYTE
ADRVIQRTNSMQQLEQWIKIQKGRGHEEETRGVISYQTLPRNMPSHRAQI
MARYPEGYRTLPRNSKTRPESICSVTPSTHDKTLGPGAEEKRRSMRDDTM
WQLYEWQQRQFYNKQSTLPRHSTLSSPKTMVNISDQTMHSIPTSPSHGSI
AAYQGYSPQRTYRSEVSSPIQRGDVTIDRRHRAHHPKHVYVPDRRSVPAG
LTLQSVSPQSLQGKTLSQDEGRGTLYKYRPEEVDIDAKLSRLCEQDKVVH
ALEEKLQQLHKEKYTLEQALLSASQEIEMHADNPAAIQTVVLQRDDLQNG
LLSTCRELSRATAELERAWREYDKLEYDVTVTRNQMQEQLDHLGEVQTES
AGIQRAQIQKELWRIQDVMEGLSKHKQQRGTTEIGMIGSKPFSTVKYKNE
GPDYRLYKSEPELTTVAEVDESNGEEKSEPVSEIETSVVKGSHFPVGVVP
PRAKSPTPESSTIASYVTLRKTKKMMDLRTERPRSAVEQLCLAESTRPRM
TVEEQMERIRRHQQACLREKKKGLNVIGASDQSPLQSPSNLRDNPFRTTQ
TRRRDDKELDTAIRENDVKPDHETPATEIVQLKETEPQNVDFSKELKKTE
NISYEMLFEPEPNGVNSVEMMDKERNKDKMPEDVTFSPQDETQTANHKPE
EHPEENTKNSVDEQEETVISYESTPEVSRGNQTMAVKSLSPSPESSASPV
PSTQPQLTEGSHFMCV

(alternative human PEPP2 sequence; possibly a splice variant with a longer C-terminal region; see also Accession No AF302150)

or

MSNKTGGKRPATTNSDIPNHNMVSEVPPERPSVRATRTARKAIAFGKRSH
SMKRNPNAPVTKAGWLFKQASSGVKQWNKRWFVLVDRCLFYYKDEKEESI
LGSIPLLSFRVAAVQPSDNISRKHTFKAEHAGVRTYFFSAESPEEQEAWI
QAMGEAARVQIPPAQKSVPQAVRHSHEKPDSENVPPSKHHQQPPHNSLPK
PEPEAKTRGEGDGRGCEKAERRPERPEVKKEPPVKANGLPAGPEPASEPG
SPYPEGPRVPGGGEQPAQPNGWQYHSPSRPGSTAFPSQDGETGGHRRSFP
PRTNPDKIAQRKSSMNQLQQWVNLRRGVPPPEDLRSPSRFYPVSRRVPEY
YGPYSSQYPDDYQYYPPGVRPESICSMPAYDRISPPWALEDKRHAFRNGG
GPAYQLREWKEPASYGRQDATVWIPSPSRQPVYYDELDAASSSLRRLSLQ
PRSHSVPRSPSQGSYSRARIYSPVRSPSARFERLPPRSEDIYADPAAYVM
RRSISSPKVPPYPEVFRDSLHTYKLNEQDTDKLLGKLCEQNKVVREQDRL
VQQLRAEKESLESALMGTHQELEMFGSQPAYPEKLRHKKDSLQNQLINIR
VELSQATTALTNSTIEYEHLESEVSALHDDLWEQLNLDTQNEVLNRQIQK
EIWRIQDVMEGLRKNNPSRGTDTAKHRGGLGPSATYSSNSPASPLSSASL

TSPLSPFSLVSGSQGSPTKPGSNEPKANYEQSKKDPHQTLPLDTPRDISL
VPTRQEVEAEKQAALNKVGVVPPRTKSPTDDEVTPSAVVRRNASGLTNGL
SSQERPKSAVFPGEGKVKMSVEEQIDRMRRHQSGSMKEKRRSLQLPASPA
PDPSPRPAYKVVRRHRSIHEVDISNLEAALRAEEPGGHAYETPREEIARL
RKMELEPQHYDVDINKELSTPDKVLIPERYIDLEPDTPLSPEELKEKQKK
VERIKTLIAKSSMQNVVPIGEGDSVDVPQDSESQLQEQEKRIEISCALAT
EASRRGRMLSVQCATPSPPTSPASPAPPANPLSSESPRGADSSYTMRV

(human PEPP3 amino acid sequence)

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

A further aspect of the invention provides a substantially pure polypeptide comprising the amino acid sequence

MEGVLYKWTNYLTGWQPRWFVLDNGILSYYDSQDDVCKGSKGSIKMAVCE
IKVHSADNTRMELIIPGEQHFYMKAVNAAERQRWLVALGSSKACLTDTRT
KKEKEISETSESLKTKMSELRLYCDLLMQQVHTIQEFVHHDENHSSPSAE
NMNEASSLLSATCNTFITTLEECVKIANAKFKPEMFQLHHPDPLVSPVSP
SPVQMMKRSVSHPGSCSSERSSHSIKEPVSTLHRLSQRRRRTYSDTDSCS
DIPLEDPDRPVHCSKNTLNGDLASATIPEESRLTAKKQSESEDTLPSFSS

(human FAPP1 amino acid sequence; see also Accession No AF286162)

or

MEGVLYKWTNYLTGWQPRWFVLDNGILSYYDSQDDVCKGSKGSIKMAVCE
IKVHSADNTRMELIIPGEQHFYMKAVNAAERQRWLVALGSSKACLTDTRT
KKEKEISETSESLKTKMSELRLYCDLLMQQVHTIQEFVHHDENHSSPSAE
NMNEASSLLSATCNTFITTLEECVKIANAKFKPEMFQLHHPDPLVSPVSP
SPVQMMKRSVSHPGSCSSERSSHSIKEPVSTLHRLSQRRRRTYSDTDSCS
DIPLEDPDRPVHCSKNTLNGDLASATIPEESRLTAKKQSESEDTLPSFSS

(mouse FAPP1 amino acid sequence; see also Accession No AF286163)

or

MEGVLYKWTNYLSGWQPRWFLLCGGILSYYDSPEDAWKGCKGSIQMAVCE
IQVHSVDNTRMDLIIPGEQYFYLKARSVAERQRWLVALGSAKACLTDSRT
QKEKEFAENTENLKTKMSELRLYCDLLVQQVDKTKEVTTTGVSNSEEGID
VGTLLKSTCNTFLKTLEECMQIANAAFTSELLYHTPPGSPQLAMLKSSKM
KHPIIPIHNSLERQTELSTCENGSLNMEINGEEEILMKNKNSLYLKSAEI

DCSISSEENTDDNITVQGEIMKEDRMENLKNHDNNLSQSGSDSSCSPECL
WEEGKEVIPTFFSTMNTSFSDIELLEDSGIPTEAFLASCCAVVPVLDKLG
PTVFAPVKMDLVENIKKVNQKYITNKEEFTTLQKIVLHEVEADVAQVRNS
ATEALLWLKRGLKFLKGFLTEVKNGEKDIQTALNNAYGKTLRQHHGWVVR
GVFALALRATPSYEDFVAALTVKEGDHRKEAFSIGMQRDLSLYLPAMKKQ
MAILDAL*

(human FAPP2 amino acid sequence; see also Accession No AF380162)
or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

A further aspect of the invention provides a substantially pure polypeptide comprising the amino acid sequence

DVRAMLRGSRLRKIRSRTWHKERLYRLQED

or

FEGTLYKRGALLKGWKPRWFVLNVT (PH30)

or

RPGLRALKKMGLTEDEDEDVRAMLRGSRLRKIRSRTWHKERLYRLQEDGL
SVWFQRRIPRAPSQHIFFVQHIEAVREGHQSEGLRRFGGAFAPARCLTIA
FKGRRKNLDLAAPTAEEAQRWVRGLTKLRARLDAMSQRERLDHWIHSYLH
RADSNQDSKMSFKEIKSLLRILV

(PH83)

or

KEGNLKKKGGGEGGRNWTVRWFKLKND

(*Dictyostelium* PH domain polypeptide)
or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof. It is preferred that the polypeptide comprises a PH domain, still more preferably a PH domain that has at least five of the six resïdues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM). Still more preferably, the PH domain is capable of binding to a phosphoinositide.

Standard IUPAC one and three letter codes are used for amino acid sequences used in the specification, and the amino acid sequences are listed N-terminal to C-terminal as is conventional.

By "substantially pure" we mean that the said polypeptide is substantially free of other proteins. Thus, we include any composition that includes at least 30% of the protein content by weight as the said polypeptide, preferably at least 50%, more preferably at least 70%, still more preferably at least 90% and most preferably at least 95% of the protein content is the said polypeptide.

Thus, the invention also includes compositions comprising the said polypeptide and a contaminant wherein the contaminant comprises less than 70% of the composition by weight, preferably less than 50% of the composition, more preferably less than 30% of the composition, still more preferably less than 10% of the composition and most preferably less than 5% of the composition by weight.

The invention also includes the substantially pure said polypeptide when combined with other components *ex vivo*, said other components not being all of the components found in the cell in which said polypeptide is found. As is described below, the polypeptides of the invention can be produced using recombinant DNA technology.

Variants (whether naturally-occurring or otherwise) may be made using the methods of protein engineering and site-directed mutagenesis well known in the art using the recombinant polynucleotides described below.

By "fragment of said polypeptide" we include any fragment which retains activity or which is useful in some other way, for example, for use in raising antibodies or in a binding or other assay, or which fragment may have other functions as described in more detail below. Preferred fragments of TAPP are discussed further below.

By "fusion of said polypeptide" we include said polypeptide fused to any other polypeptide. For example, the said polypeptide may be fused to a polypeptide such as glutathione-S-transferase (GST) or protein A in order to facilitate purification of said polypeptide. Examples of such fusions are well known to those skilled in the art. Similarly, the said polypeptide may be fused to an oligo-histidine tag such as His6 or to an epitope recognised by an antibody such as the well known Myc tag epitope. Fusions to any variant, fragment or derivative of said polypeptide are also included in the scope of the invention. It will be appreciated that fusions (or variants, fragments, derivatives or fusions thereof) which retain desirable properties, such as binding properties (for example, the ability to bind to a particular phosphoinositide or interacting polypeptide) or the ability to change sub-cellular location in response to stress, insulin or growth factor signalling (in an intact cell) or other biological functions, of the said polypeptide (for example TAPP, PEPP or FAPP) are particularly preferred. It is also particularly preferred if the fusions are one which are suitable for use in the screening assays described earlier.

It will be appreciated that fusions which retain desirable properties, such as binding properties or other biological functions, of the said polypeptide are particularly preferred. It is also particularly preferred if the fusions are one which are suitable for use in the screening assays described above. It will be appreciated that before the present invention, no requirement for

producing any of the said polypeptides, or for variants or fusions or derivatives thereof, had not been appreciated in the art since their involvement in phosphoinositide signalling was not known. In particular it was not appreciated that the said polypeptides and variants and fusions thereof would be useful in screening methods for drugs and drug-like compounds.

By "variants" of the polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative. In particular we include variants of the polypeptide where such changes do not substantially alter the activity of the said polypeptide. In particular we include variants of the polypeptide where such changes do not substantially alter the activity, for example the binding activity (for example to a phosphoinositide) of the said polypeptide. Variants of the said polypeptides do not include polypeptides which have the amino acid sequence of known polypeptides comprising a PH domain.

It will be appreciated that a variant that comprises substantially all of the sequence shown above (for example substantially full-length TAPP, PEPP or FAPP) may be particularly useful. By "substantially all" is meant at least 80%, preferably 90%, still more preferably 95%, 98% or 100% (ie all) of the said sequence. By "substantially full-length" is meant comprising at least 80%, preferably 90%, still more preferably 95%, 98% or 100% (ie all) of the sequence of the full length polypeptide.

By "conservative substitutions" is intended combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr.

It is particularly preferred if the polypeptide variant has an amino acid sequence which has at least 65% identity with either amino acid sequence given above, more preferably at least 75%, still more preferably at least 90%, yet more preferably at least 95%, and most preferably at least 98% or 99% identity with the appropriate amino acid sequence given above, most preferably with the amino acid sequence given above for human TAPP, PEPP or FAPP.

It is particularly preferred if the polypeptide variant has an amino acid sequence which has at least 90% identity with the amino acid sequence given above, more preferably at least 92%, still more preferably at least 95%, yet more preferably at least 96%, and most preferably at least 98% or 99% identity with the amino acid sequence given above.

The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequences have been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994), Clustal-W - improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nuc. Acid Res.* **22**, 4673-4680).

The parameters used may be as follows:

Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.

Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.

Scoring matrix: BLOSUM.

"Fragments" and "variants" also include those which are useful to prepare antibodies which will specifically bind the said polypeptide or mutant forms thereof lacking the function of the native polypeptide. Such variants and fragments will usually include at least one region of at least five consecutive amino acids which has at least 90% homology with the most homologous five or more consecutive amino acids region of the said polypeptide (ie when comparing forms of the polypeptide from different species). A fragment is less than 100% of the whole polypeptide.

The following peptides may be useful as TAPP1 (particularly human TAPP1) immunogens: YVDRQNRICGFLDIEENENSGK (this one would also be expected to recognise TAPP2) and RYTSRAGECSTYVGSHANVPS.

The following peptides may be useful as TAPP2 (particularly mouse TAPP2) immunogens: RVRHRSEPQHPKEKPFVFNL and KRGLCKAPSVASSWQPWTPVKK.

The amino acid sequences of TAPP1 and TAPP2 are most dissimilar in the C-terminal region (excluding the extreme C-terminus), as is apparent from Figure 3A. Accordingly, a peptide with a sequence derived from the less-conserved C-terminal region of TAPP1 or TAPP2 may be useful in

preparing antibodies that are specific for TAPP1 or TAPP2, respectively. A peptide with a sequence derived from the more conserved N-terminal region of TAPP1/TAPP2 may be useful in preparing antibodies that react with both TAPP1 and TAPP2.

It will be recognised by those skilled in the art that the polypeptide of the invention may be modified by known polypeptide modification techniques. These include the techniques disclosed in US Patent No 4,302,386 issued 24 November 1981 to Stevens, incorporated herein by reference. Such modifications may enhance the immunogenicity of the antigen, or they may have no effect on such immunogenicity. For example, a few amino acid residues may be changed. Alternatively, the antigen of the invention may contain one or more amino acid sequences that are not necessary to its immunogenicity. Unwanted sequences can be removed by techniques well known in the art. For example, the sequences can be removed via limited proteolytic digestion using enzymes such as trypsin or papain or related proteolytic enzymes.

Alternatively, smaller polypeptides corresponding to antigenic parts of the polypeptide may be chemically synthesised by methods well known in the art. These include the methods disclosed in US Patent No 4,290,944 issued 22 September 1981 to Goldberg, incorporated herein by reference.

Thus, the polypeptide of the invention includes a class of modified polypeptides, including synthetically derived polypeptides or fragments of the original polypeptide, having common elements of origin, structure, and immunogenicity that are within the scope of the present invention.

An additional embodiment of this aspect of the invention relates to a peptide

or polypeptide which has the amino acid sequence of an epitope-bearing portion of a polypeptide of the invention, ie having an amino acid sequence described above. Such peptides or polypeptides include portions of a polypeptide of the invention with at least six or seven, preferably at least nine, and more preferably at least about 30 amino acids to about 50 amino acids, although epitope-bearing polypeptides of any length up to and including the complete amino acid sequence of a polypeptide of the invention described above also are included in the invention.

A particular embodiment of the invention provides a substantially pure TAPP polypeptide which consists of the amino acid sequence indicated above for human or mouse TAPP1 or TAPP2 or naturally occurring allelic variants thereof.

A preferred fragment of the TAPP polypeptide of the invention comprises the amino acid sequence of amino acids 1 to 147 of any of the given TAPP amino acid sequences, preferably of the given amino acid sequence for human TAPP1. This fragment comprises the N-terminal PH domain of TAPP. It is further preferred that the fragment does not comprise the amino acid sequence of about amino acids 190 to about 290 of the given amino acid sequence of TAPP. This fragment comprises the N-terminal PH domain of TAPP1 and does not comprise the C-terminal PH domain of TAPP1.

A further preferred fragment of the polypeptide of the invention comprises the amino acid sequence of amino acids 95 to 404 of any of the given TAPP amino acid sequences, preferably of the given amino acid sequence for human TAPP1. This fragment comprises the C-terminal PH domain of TAPP1. It is further preferred that the fragment does not comprise the

amino acid sequence of about amino acids 10 to 111 of the given amino acid sequence. This fragment comprises the C-terminal PH domain of TAPP1 and does not comprise the entire N-terminal domain of TAPP1.

A particular embodiment of the invention provides a substantially pure PEPP polypeptide which consists of the amino acid sequence indicated above for human or mouse PEPP1, PEPP2 or PEPP3 or naturally occurring allelic (including splice) variants thereof.

A particular embodiment of the invention provides a substantially pure FAPP polypeptide which consists of the amino acid sequence indicated above for human or mouse FAPP1 or FAPP2 or naturally occurring allelic variants thereof.

Further preferred fragments of TAPP, PEPP and FAPP (for example fragments comprising PH domains) are discussed in Example 1, for example in the section relation to cloning of PH domains and in Figure 1.

Preferred fusions of these fragments include fusions as described in Example 1, for example fusions in which the said fragment has an N-terminal GST tag followed by a myc epitope tag or a FLAG (DYKDDDDK) epitope tag fused to the N-terminus of the said fragment.

A variant of the TAPP polypeptide of the invention which may be useful is a variant (or fragment, derivative or fusion of such a variant) wherein the residue equivalent to Arg212 of the given human TAPP1 amino acid sequence is mutated, for example to a leucine residue. Such a variant may be less able or unable to bind to PtdIns(3,4)P_2 (or other phosphoinositide), as described in Example 1.

Other variants of the polypeptide of the invention which may be useful are variants (or fragments, derivatives or fusions of such a variant) wherein the residue equivalent to any of the lysine or arginine residues of the PPBP is mutated to an acidic residue, for example glutamate or to a large hydrophobic residue, for example methionine. Such a variant may be less able or unable to bind to a phosphoinositide, as described in Example 1.

It will be appreciated that such fragments and variants may be useful in screening assays, medicine and/or in investigating the involvement of TAPP or other polypeptide of the invention in normal and diseased cells.

Thus, for example, it will be appreciated that a fragment of TAPP comprising the N-terminal (putative protein-binding) PH domain but not the C-terminal (phosphoinositide-binding) PH domain or a fragment of TAPP comprising the N-terminal PH domain but not the C-terminal PH domain may be capable of acting as an inhibitor, for example a dominant-negative inhibitor, of signalling *via* a signalling pathway in which TAPP may be involved, as discussed further below, for example signalling *via* an integrin receptor or a growth factor receptor. A variant of TAPP in which any of the conserved Lys/Arg sites in the PPBM is replaced with an acidic or hydrophobic residue, for example leucine, may act as a dominant negative mutant, which may bind to interacting polypeptides (for example *via* the N-terminal PH domain) but not to the phosphoinositide. Thus, such a fragment may be useful, for example, as an anti-cancer agent or in the promotion of apoptosis. Promotion of apoptosis may be beneficial in the resolution of inflammation. Inhibition of TAPP activity may inhibit platelet activation, which may be useful in reducing or preventing thrombosis. This may be important in patients at risk of thrombosis (for example obese

patients or those with a history of thrombosis) and/or before, during or after surgery.

Over-expression of a substantially full-length native said polypeptide, for example a TAPP, PEPP or FAPP polypeptide may be useful in increasing signalling in which the said polypeptide is involved and therefore may also be useful in the treatment of diabetes or defects of glycogen regulation. It may also be useful in reducing apoptosis; thus, it may be useful in treating a patient in need of protection against apoptosis. Reducing apoptosis may be useful following ischaemic injury, for example stroke or myocardial infarction, and in tissue repair. It may also be useful in the treatment of patient before, after or during heart surgery.

It will be appreciated that a fusion of a polypeptide, variant or fragment of the invention wherein the fusion comprises a GST and/or FLAG or myc epitope portion may be particularly useful. For example, a GST tag may be useful in purifying or detecting the fusion protein, as described in Example 1, for example in detecting the interaction between the fusion protein and a phospholipid.

It is particularly preferred, although not essential, that the variant or fragment or derivative or fusion of the said polypeptide, or the fusion of the variant or fragment or derivative has at least 30% of the PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 binding affinity of the said polypeptide, for example TAPP, PEPP or FAPP, but is not capable of binding to PtdIns(3,4,5)P_3. It is more preferred if the variant or fragment or derivative or fusion of the said polypeptide, or the fusion of the variant or fragment or derivative has at least 50%, preferably at least 70% and more preferably at least 90% of the phosphoinositide binding activity of the said polypeptide,

for example TAPP, PEPP or FAPP. However, it will be appreciated that variants or fusions or derivatives or fragments which are devoid of one or more binding activities as set out above may nevertheless be useful, for example as described above or by interacting with another polypeptide, or as antigens in raising antibodies. Methods of measuring the binding affinity with phosphoinositides are described, for example, in Example 1 below. Methods of measuring protein-protein interactions are well known to those skilled in the art and are discussed above.

By "residue equivalent to" a particular residue, for example the residue equivalent to Arg212 of human TAPP1, is included the meaning that the amino acid residue occupies a position in the native two or three dimensional structure of a polypeptide corresponding to the position occupied by the said particular residue, for example Arg212, in the native two or three dimensional structure of full-length human TAPP1.

The residue equivalent to a particular residue, for example Arg212 of full-length human TAPP1, may be identified by alignment of the sequence of the polypeptide with that of full-length human TAPP1 in such a way as to maximise the match between the sequences. The alignment may be carried out by visual inspection and/or by the use of suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group, which will also allow the percent identity of the polypeptides to be calculated, or using the Align program (Pearson (1994) in: Methods in Molecular Biology, Computer Analysis of Sequence Data, Part II (Griffin, AM and Griffin, HG eds) pp 365-389, Humana Press, Clifton). Thus, residues identified in this manner are also "equivalent residues".

It will be appreciated that in the case of truncated forms of human TAPP1 or in forms where simple replacements of amino acids have occurred it is facile to identify the "equivalent residue".

Peptides may be synthesised by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lu *et al* (1981) *J. Org. Chem.* **46**, 3433 and references therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is effected using 20% piperidine in N,N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4'-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalising agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N,N-dicyclohexyl-carbodiimide/1-hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic

acid containing a 50% scavenger mix. Scavengers commonly used are ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesised. Trifluoroacetic acid is removed by evaporation *in vacuo*, with subsequent trituration with diethyl ether affording the crude peptide. Any scavengers present are removed by a simple extraction procedure which on lyophilisation of the aqueous phase affords the crude peptide free of scavengers. Reagents for peptide synthesis are generally available from Calbiochem-Novabiochem (UK) Ltd, Nottingham NG7 2QJ, UK. Purification may be effected by any one, or a combination of, techniques such as size exclusion chromatography, ion-exchange chromatography and (principally) reverse-phase high performance liquid chromatography. Analysis of peptides may be carried out using thin layer chromatography, reverse-phase high performance liquid chromatography, amino-acid analysis after acid hydrolysis and by fast atom bombardment (FAB) mass spectrometric analysis.

A further aspect of the invention provides a recombinant polynucleotide encoding a phosphoinositide-binding polypeptide of the invention, ie a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, wherein the polypeptide is not centaurin-β2 or AtPH1[19], or encoding a variant or fragment or derivative or fusion of said polypeptide or a fusion of a said variant or fragment or derivative. Preferences and exclusions for the said polynucleotide variant are the same as in the first aspect of the invention, except that the following Expressed Sequence Tags (ESTs) are also excluded: ESTs listed in Table 3 or in Example 1; AA762924 (mouse TAPP1); T04439 (AtPH1 from *Arabidopsis thaliana*); AA967911 (mouse centaurin-β2). The following are also excluded: AI739438; BE303674; F23241; KIAA0969 (PEPP3).

All ESTs are identified by the Genbank accession number, as described in Example 1.

A further aspect of the invention provides a recombinant polynucleotide suitable for expressing a said phosphoinositide-binding protein of the invention or suitable for expressing a variant or fragment or derivative of fusion of said polypeptide or a fusion of a said variant or fragment or derivative. Preferences and exclusions for the said polynucleotide variant are equivalent to those in relation to the said phosphoinositide-binding polypeptide of the invention.

By "suitable for expressing" is meant that the polynucleotide is a polynucleotide that may be translated to form the polypeptide, for example RNA, or that the polynucleotide (which is preferably DNA) encoding the polypeptide of the invention is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. The polynucleotide may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by any desired host; such controls may be incorporated in the expression vector.

It is not considered that the ESTs listed above are a polynucleotide as defined above; however, for the avoidance of doubt, the ESTs excluded above are further excluded from this aspect of the invention.

A further aspect of the invention is a replicable vector suitable for expressing a polypeptide as defined in the first aspect of the invention or suitable for expressing a variant or fragment or derivative of fusion of said polypeptide or a fusion of a said variant or fragment or derivative.

Preferences and exclusions for the said polynucleotide variant are equivalent to those in relation to the phosphoinositide-binding polypeptide of the invention. For example, the replicable vector may be suitable for expressing a fusion of the said phosphoinositide-binding polypeptide, in particular a GST fusion.

A further aspect of the invention is a polynucleotide encoding a fusion of the said phosphoinositide-binding polypeptide of the invention, or a fusion of a variant or fragment or derivative, in particular a GST fusion. A still further aspect is a vector suitable for replication in a eukaryotic, preferably mammalian, cell, comprising a polynucleotide encoding the polypeptide, or a variant or fragment or derivative or a fusion of the polypeptide, as defined in the first aspect of the invention, or a fusion of a variant or fragment or derivative, in particular a GST fusion. Any of the EST clones listed above as excluded from the polynucleotide of the invention which are vectors which may be suitable for replication in a mammalian/eukaryotic cell are excluded from this aspect of the invention.

Characteristics of vectors suitable for replication in mammalian/eukaryotic cells are well known to those skilled in the art. It will be appreciated that a vector may be suitable for replication in both prokaryotic and eukaryotic cells.

In one preferred embodiment the polynucleotide comprises the nucleotide sequence:

TTTGGTGCAGTTTAGCATGTTCCTCTGTGTTCTGCATCTCCTGTAGTGTA
ATGTTCAAGCTCAGAAATGCCTTATGTGGATCGTCAGAATCGCATTTGTG
GTTTTCTAGACATTGAAGAAAATGAAAACAGTGGGAAATTTCTTCGAAGG
TACTTCATACTGGATACCAGAGAAGATAGTTTCGTGTGGTACATGGATAA
TCCACAGAACCTACCTTCTGGATCATCACGTGTTGGAGCCATTAAGCTTA

CCTACATTTCAAAGGTTAGCGATGCTACTAAGCTAAGGCCAAAGGCGGAG
TTCTGTTTTGTTATGAATGCAGGAATGAGGAAGTACTTCCTACAAGCCAA
TGATCAGCAGGACCTAGTGGAATGGGTAAATGTGTTAAACAAAGCTATAA
AAATTACAGTACCAAAGCAGTCAGACTCACAGCCTAATTCTGATAACCTA
AGTCGCCATGGTGAATGTGGGAAAAAGCAAGTGTCTTACAGAACTGATAT
TGTTGGTGGCGTACCCATCATTACTCCCACTCAGAAAGAAGAAGTAAATG
AATGTGGTGAAAGTATTGACAGAAATAATCTGAAACGGTCACAAAGCCAT
CTTCCTTACTTTACTCCTAAACCACCTCAAGATAGTGCGGTTATCAAAGC
TGGATATTGTGTAAAACAAGGAGCAGTGATGAAAAACTGGAAGAGAAGAT
ATTTTCAATTGGATGAAAACACAATAGGCTACTTCAAATCTGAACTGGAA
AAGGAACCTCTTCGCGTAATACCACTTAAAGAGGTTCATAAAGTCCAGGA
ATGTAAGCAAAGCGACATAATGATGAGGGACAACCTCTTTGAAATTGTAA
CAACGTCTCGAACTTTCTATGTGCAGGCTGATAGCCCTGAAGAGATGCAC
AGTTGGATTAAAGCAGTCTCTGGCGCCATTGTAGCACAGCGGGGTCCCGG
CAGATCTGCGTCTTCTGAGCATCCCCCCGGTCCTTCAGAATCCAAACACG
CTTTCCGTCCTACCAACGCAGCCGCCGCCACCTCACATTCCACAGCCTCT
CGCAGCAACTCTTTGGTCTCAACCTTTACCATGGAGAAGCGAGGATTTTA
CGAGTCTCTTGCCAAGGTCAAGCCAGGGAACTTCAAGGTCCAGACTGTCT
CTCCAAGAGAACCAGCTTCCAAAGTGACTGAACAAGCTCTGTTAAGACCT
CAAAGTAAAAATGGCCCTCAGGAAAAAGATTGTGACCTAGTAGACTTGGA
CGATGCGAGCCTTCCGGTCAGTGACGTGTGAGGCAGAAGCGCACGGAGCC
TGCCTGCCTCTGCCGTCCTCAGTTACCTTTCATGAGGCTTCTAGCCAAAG
ATGATAAAGGGGGAAATGGTTTTTAGTGCGTATATTATACTGCCTCTTAG
GTGTACTCTT

(human TAPP1)

or

CGAGGGGAGCGAGAGGCGCGGAGAGTTTGGCAGGCAGACCCAGAAATCCC
TGGAGCGCGGCGGACCCGGCGGCCGGAGGGGCGACCCCGCCCGATGTAac
GCGCCCCGCCCGAGCCCCGGCCCCTGCaCGGGGGGGGGTGATGTGAGCAG
AGCCCAGGAATGCCTTATGTGGATCGGCAGAACCGAATCTGTGGGTTTCT
GGACATCGAGGAGCATGAGAACAGCGGCAAGTTTCTGCGGAGGTACTTCA
TTCTGGACACCCAGGCTAACTGCCTCCTCTGGTATATGGACAACCCCCAG
AATCTGGCAATGGGGGCAGGAGCTGTTGGAGCTTTGCAGCTGACCTACAT
CTCGAAGGTGAGCATAGCTACCCCAAAACAGAAACCAAAAACTCCATTTT
GCTTTGTTATCAATGCCCTGTCTCAGAGATATTTCCTTCAAGCCAATGAT
CAGAAAGATATGAAGGACTGGGTTGAAGCCCTGAACCAAGCCAGCAAGAT
CACCGTTCCCaAAGGTGGGGGCCTACCCATGACCACTGAAGTTCTCAAGA
GCTTAgCAGCTCCTCCAGCCCTGGAGAAgAAgCCACAGGTGGCCTACAAG
ACGGAGATCATTGGAGGGGTGGTGGTCCACACACCCATCAGCCAGAACGG

TGGGGATGGGCAGGAAGGGAGTGAGCCCGGGTCCCACACCATCCTTcGAA
GGTcTCAGAGTTACATCCCCACGTCAGGCTGCCGTGCTTCCACTGGGCCT
CCCCTCATTAAGAGTGGTTACTGCGTGAAGCAAGGGAATGTGCGGAAGAG
CTGGAAACGTCGcTTcTTTGCACTTGATGACTTTACCATCTGCTACTTCA
AGTGTGAGCAGGACCGAGAACCACTGCGCACCATATTTTTTAAGGATGTT
cTGAAGACCCATGAATGTCTGGTCAAGTCTGGTGATCTcTTAATGAGGGA
CAACCTGTTTGAAATaaTAACAAGCTCCAGGACCTTCTACGTACAGGCAG
ACAGTCCAGAAGACATGCACAGCTGGATTAAGGAGATTGGCGCAGCTGTC
CAGGCCCTCAAGTGCCACCCC

(partial human TAPP2)

or

ATGCCTtaTGTGGATCGACAGAATCGCATCTGTGGaTTTCTAGACATTGA
AGAAAATGAGAACAGTGGGAAATTTCTTCGACGGTATTTCATCCTGGATA
CCAGAGAAGACAGCTTTGTATGGTACATGGATAATCCACAGnnnnnnnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnATGCA
GGAATGAGAAAATACTTTCTACAAGCTAATGATCAGCAGGACTTAGTGGA
GTGGGTAAATGTCTTGAACAAAGCTATAAAAATTACAGTACCAAAGCAGT
CAGACTCACAGCCGGCCTCCGACAGCCTGAGTCGCCAAGGTGACTGTGGT
AAGAAGCAAGTGTCTTACAGAACTGACATTGTTGGTGGTGTGCCCATCAT
CACGCCGACGCAGAAAGAAGAAGTAAACGAATGTGGTGAGAGTCTGGATA
GAAACAATTTGAAACGGTCACAGAGCCATCTTCCTTACTTTGCTCCTAAG
CCACCTTCAGACAGTGCAGTTATCAAAGCTGGGTATTGTGTGAAGCAAGG
AGCGGTGATGAAAAACTGGAAGAGAAGATATTTTCAATTGGATGAAAACA
CAATAGGCTACTTCAAATCTGAACTGGAGAAGGAACCTCTGCGGGTGATA
CCACTTAAAGAAGTGCACAAAGTCCAGGAGTGCAAACAGAGTGACATAAT
GATGAGGGACAACCTGTTTGAAATCGTGACGACATCTCGGACTTTCTATG
TGCAGGCTGATAGCCCTGAAGAGATGCACAGTTGGATTAAAGCAGTCTCT
GGCGCCATCGTAGCACAGCGGGGACCTGGCAGGTCATCCTCTTCTnnnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn

(partial mouse TAPP1; the run of n's indicates a gap of unknown length)

or

CCACGCGTCCGGCGGCGAAACTTCTCCGAGGTTCAAGCACAGGGGTGGTA
GCCCCTCAAGGACTGCCCGGGCAGCGGGTATGGGAGGAGCGCA*AGAACG
TCCCAGGGTGATGTGAACAGAGCCCAGGAATGCCTTATGTGGATCGGCAG
AACCGAATCTGTGGGTTTCTGGATATTGAAGACAATGAGAACAGTGGCAA
ATTCCTCCGGAGATACTTTATCCTGGATACCCAGGCCAACTGCCTCCTCT
GGTACATGGACAATCCCCAAAACCTGGCCGTTGGGGCAGGAGCTGTCGGA

TCTCTGCAGCTGACCTACATCTCGAAGGTGAGCATAGCTACCCCAAAGCA
GAAACCTAAAACGCCATTCTGCTTCGTTATCAATGCCCTGTCTCAGAGAT
ATTTTCTTCAAGCCAATGACCAGAAAGATCTGAAGGACTGGGTAGAAGCC
TTGAACCAAGCCAGCAAGATCACTGTACCCAAAGCTGGGACAGTACCCTT
GGCCACAGAAGTTCTCAAAAACTTAACAGCTCCTCCCACCCTAGAGAAGA
AGCCGCAGGTGGCCTACAAGACTGAGATCATCGGGGGTGTGGTGGTACAA
ACGCCTATCAGCCAGAACGGTGGGGATGGGCAGGAAGGGTGCGAGCCAGG
GACTCACGCCTTCCTGCGAAGGTCTCAGAGCTACATCCCCACGTCAGGCT
GCCGCCCTTCCACTGGGCCTCCCCTCATTAAGAGTGGCTACTGTGTGAAG
CAAGGGAATGTGCGGAAGAGTTGGAAACGACGCTTCTTTGCCCTCGATGA
CTTTACCATCTGCTACTTCAAGTGTGAGCAGGACAGAGAGCCTCTGCGTA
CCATACCGCTCAAGGATGTTCTCAAGACTCACGAGTGTCTGGTCAAGTCT
GGTGATCTCTTAATGAGGGACAACCTGTTTGAAATCATAACCACCTCCAG
GACGTTCTACGTACAGGCGGACAGCCCTGAGGACATGCACAGCTGGATCG
AGGGGATTGGAGCAGCTGTCCAGGCTCTGAAGTGCCACCCTAGGGAGCCG
TCCTTTTCAAGGTCCATTTCTTTGACTCGACCTGGAAGTTCTACCCTTAC
AAGCGCGCCTAACTCCATCTTGTCAAGAAGGCGGCCACCAGCAGAAGAGA
AAAGAGGTCTCTGTAAGGCCCCTTCGGTGGCCTCCTCCTGGCAACCCTGG
ACACCTGTCCCCCAGGCTGAGGAAAAGCCGTTGTCGGTGGAGCATGCTCC
AGAGGACTCTCTATTCATGCCTAACCCTGGGGAGAGCACAGCTACAGGGG
TGCTGGCAAGTTCTCGAGTCAGGCACAGGTCGGAGCCCCAGCACCCCAAG
GAGAAGCCATTTGTATTCAACCTTGATGACGAAAACATACGAACCTCTGA
TGTGTGATATGCAGTGCCCGTTGCGTGCAGGAGAGCCAGGGGCTGTGACT
TATTTTCTCTGCCATGGTAGAGGACAGAGTCTAATGGCACTCACAGTGGA
GGGGCTCGTCTAGCTGGCTTGGTTTGCTATTATTGACACCATTTATTTAA
CTGGG

(mouse TAPP2)

or

AAACTGGGAGAGGGAGGAAGGGAGAAAGTGAGAAGGGAAATCGGAAAGAG
AAAAGGGAGGAAACGGCAGAGCCAGAGAGAAAGAGGAAGAGACTGAGTGT
GAAGGAGAGAGGACACAGGGGATGACTGAGAGACAGAGAGAGAGAGAGAG
AGAGAATGAGACAGAGACTTAAGGAAGAGACCCTGTGAGTCTGACAATAA
AAGATTTGGACAGAAACAGAAAGATTGGAGAGAGAGAGAGAGGGAGAGAA
TGAGTGAGAGAGAGACTGGAAGAGACAGAGATCAGAGGGAGACACAGAAA
GTGAGAGTGGGGAGAGAGGTAGTGTAAAAGGAAGAGAGAGAGAGAGAGAC
CGTAAGAGACAGGAGACAAAGAGACAAAAAGTGTGAGTGAGCAGGTGAGG
AGAGAGATTGAGAACTATGAGAGACAGCAGCTAAGAGACAAAGGAGGCGG
GAGACTGCCTAGGTGCCGCAGCACCCACACCGTCCTCTTGCCCCCCCGTC
ACTGGGACCCCAGAGCTGGCCCTTGATGGAGGGGAGCCGACCTCGCAGCA
GCCTGAGCCTGGCCAGCAGCGCCTCCACCATCTCCTCGCTCAGCAGCCTG
AGCCCCAAGAAGCCCACCCGGGCAGTAAACAAGATCCACGCCTTTGGGAA

GAGAGGCAATGCGCTCAGGAGGGATCCCAACCTTCCCGTGCACATCCGAG
GCTGGCTTCATAAGCAGGACAGCTCGGGGCTCCGTCTCTGGAAACGCCGC
TGGTTCGTCCTCTCCGGCCATTGCCTCTTTTATTACAAGGACAGCCGCGA
GGAGAGTGTCCTAGGCAGCGTCCTGCTCCCCAGCTACAATATTAGACCAG
ATGGGCCGGGAGCCCCCCGAGGGCGGCGCTTCACCTTCACCGCAGAGCAC
CCGGGCATGAGGACCTACGTTTTGGCCGCTGACACCTTAGAAGACCTGCG
GGGCTGGCTACGGGCGCTGGGCCGGGCCTCCCGTGCGGAGGGGGACGACT
ATGGGCAACCCAGGTCACCTGCACGACCCCAGCCCGGGGAGGGCCCCGGC
GGCCCCGGTGGTCCCCCGGAGGTGAGCAGAGGGGAAGAGGGGCGCATCTC
AGAATCACCGGAAGTGACTCGACTCTCCAGAGGTCGTGGTAGACCCAGGC
TGCTCACTCCCAGCCCCACAACCGACCTCCACTCTGGACTCCAGATGCGG
AGGGCGAGGAGCCCCGACCTGTTCACCCCCCTCTCTCGCCCTCCCTCGCC
TCTGAGCCTCCCCCGTCCCCGTTCTGCCCCTGCGCGGCGACCCCCTGCCC
CCTCAgGAGACACAGC

(partial human PEPP1)

or

AAACTGGGAGAGGGAGGAAGGGAGAAAGTGAGAAGGGAAATCGGAAAGAG
AAAAGGGAGGAAACGGCAGAGCCAGAGAGAAAGAGGAAGAGACTGAGTGT
GAAGGAGAGAGGACACAGGGGATGACTGAGAGACAGAGAGAGAGAGAGAG
AGAGAATGAGACAGAGACTTAAGGAAGAGACCCTGTGAGTCTGACAATAA
AAGATTTGGACAGAAACAGAAAGATTGGAGAGAGAGAGAGAGGGAGAGAA
TGAGTGAGAGAGAGACTGGAAGAGACAGAGATCAGAGGGAGACACAGAAA
GTGAGAGTGGGGAGAGAGGTAGTGTAAAAGGAAGAGAGAGAGAGAGAGAC
CGTAAGAGACAGGAGACAAAGAGACAAAAAGTGTGAGTGAGCAGGTGAGG
AGAGAGATTGAGAACTATGAGAGACAGCAGCTAAGAGACAAAGGAGGCGG
GAGACTGCCTAGGTGCCGCAGCACCCACACCGTCCTCTTGCCCCCCCGTC
ACTGGGACCCCAGAGCTGGCCCTTGATGGAGGGGAGCCGACCTCGCAGCA
GCCTGAGCCTGGCCAGCAGCGCCTCCACCATCTCCTCGCTCAGCAGCCTG
AGCCCCAAGAAGCCCACCCGGGCAGTAAACAAGATCCACGCCTTTGGGAA
GAGAGGCAATGCGCTCAGGAGGGATCCCAACCTTCCCGTGCACATCCGAG
GCTGGCTTCATAAGCAGGACAGCTCGGGGCTCCGTCTCTGGAAACGCCGC
TGGTTCGTCCTCTCCGGCCATTGCCTCTTTTATTACAAGGACAGCCGCGA
GGAGAGTGTCCTAGGCAGCGTCCTGCTCCCCAGCTACAATATTAGACCAG
ATGGGCCGGGAGCCCCCCGAGGGCGGCGCTTCACCTTCACCGCAGAGCAC
CCGGGCATGAGGACCTACGTTTTGGCCGCTGACACCTTAGAAGACCTGCG
GGGCTGGCTACGGGCGCTGGGCCGGGCCTCCCGTGCGGAGGGGGACGACT
ATGGGCAACCCAGGTCACCTGCACGACCCCAGCCCGGGGAGGGCCCCGGC
GGCCCCGGTGGTCCCCCGGAGGTGAGCAGAGGGGAAGAGGGGCGCATCTC
AGAATCACCGGAAGTGACTCGACTCTCCAGAGGTCGTGGTAGACCCAGGC
TGCTCACTCCCAGCCCCACAACCGACCTCCACTCTGGACTCCAGATGCGG
AGGGCGAGGAGCCCCGACCTGTTCACCCCCCTCTCTCGCCCTCCCTCGCC

TCTGAGCCTCCCCCGTCCCCGTTCTGCCCCTGCGCGGCGACCCCCTGCCC
CCTCAGGAGACACAGCACCCCCTGCCCGACCTCACACCCCGTTGAGTCGC
ATTGATGTCCGACCTCCTCTGGATTGGGGCCCCCAACGCCAGACCCTCTC
CCGACCCCCTACTCCCCGCCGAGGACCTCCCTCTGAGGCTGGGGGAGGAA
AGCCCCCCAGGAGTCCCCAGCACTGGAGTCAGGAGCCCAGAACACAGGCA
CACTCTGGCTCCCCCACTTATCTCCAGCTCCCCCCGCGGCCCCCTGGGAC
CCGGGCCTCCATGGTTTTATTGCCGGGTCCTCCCCTGGAGTCAACTTTCC
ACCAAAGCTTGGAGACAGATACGCTGCTGACCAAGTTGTGCGGGCAGGAC
CGGCTTCTGCGGAGGCTGCAGGAGGAGATAGACCAGAAGCAGGAGGAGAA
GGAGCAACTAGAAGCAGCTCTGGAGTTGACCCGGCAACAGCTGGGCCAAG
CCACCAGGGAGGCTGGGGCTCCCGGGAGGGCCTGGGGTCGCCAGCGCCTC
TTGCAGGACCGGCTGGTCAGTGTGAGGGCCACCCTCTGTCACTTGACTCA
GGAGCGAGAGAGGGTTTGGGACACGTACAGTGGCCTGGAGCAGGAGCTGG
GCACCTTAAGAGAGACGCTGGAGTACCTGCTGCACCTTGGTTCTCCCCAG
GACAGAGTGTCTGCTCAGCAGCAGCTGTGGATGGTGGAAGACACGCTGGC
AGGTCTGGGTGGCCCCCAGAAACCGCCCCCACACACTGAGCCTGACTCCC
CATCTCCCGTGCTCCAGGGCGAGGAGTCCTCAGAGAGGGAGAGCCTGCCA
GAGTCCTTGGAACTGAGCTCCCCTAGGTCCCCCGAGACTGACTGGGGGCG
GCCTCCTGGAGGCGACAAAGACCTCGCCAGCCCTCACTTAGGTCTTGGGT
CTCCGAGGGTCTCCCGGGCTTCCAGCCCTGAGGGTCGCCACCTCCCTTCC
CCACAGCTAGGAACCAAGGCCCCGGTGGCCCGGCCCCGGATGAATGCCCA
GGAGCAGCTGGAGCGGATGCGCAGAAACCAGGAATGTGGACGGCCCTTCC
CTCGCCCGACCTCCCCCCGGCTTCTCACCCTGGGAAGGACACTGTCCCCA
GCCAGACGCCAGCCTGACGTGGAGCAAAGGCCTGTCGTAGGACACTCGGG
AGCCCAGAAATGGCTCAGAAGCTCTGGGTCCTGGAGTAGTCCAAGGAACA
CCACCCCTTACTTGCCGACTTCCGAAGGTCACCGGGAGCGGGTTCTCAGC
CTCTCCCAAGCCCTGGCTACTGAGGCGTCGCAGTGGCACAGAATGATGAC
AGGTGGAAATTTGGACTCCCAGGGAGACCCTCTTCCCGGTGTGCCGCTGC
CTCCTTCGGACCCCACGCGCCAGGAGACCCCTCCCCCCAGATCTCCCCCG
GTGGCTAATTCGGGTTCCACGGGGTTCTCTCGCCGAGGGAGTGGGCGTGG
AGGAGGTCCCACCCCCTGGGGGCCCGCGTGGGATGCCGGGATCGCCCCTC
CGGTCCTGCCACAAGACGAGGGGGCATGGCCTCTGCGAGTCACTCTGCTA
CAATCCAGCTTGTAATCCGCCCAAAAGCGGCAGCCAATCGGAGCGCGAGG
ACGTGGTCTGGAGGTACCGCCGAAGATCTGGGACCACTCAGGGCATCAGG
GGGCGTGGTCTGGTCCCCATTGCGGGCCCGGGAGGGGAATGGTTTCTATG
GCCAAAGTTTGGTTTTCTCAACACTGTCTAAATTTGGATTAAAACTTTGA
ACTTTT

(human PEPP1)

Or

TGCAAACATCCAGTCACAGGACAACCATCACAGGACAATTGTATTTTTGT

AGTGAATGAACAGACTGTTGCAACCATGACATCTGAAGAAAAGAAGGAAC
GGCCAATAAGTATGATAAATGAAGCTTCTAACTATAACGTGACTTCAGAT
TATGCAGTGCATCCAATGAGCCCTGTAGGCAGAACTTCACGAGCTTCAAA
AAAAGTTCATAATTTTGGAAAGAGGTCAAATTCAATTAAAAGGAATCCTA
ATGCACCGGTTGTCAGACGAGGTTGGCTTTATAAACAGGACAGTACTGGC
ATGAAATTGTGGAAGAAACGCTGGTTTGTGCTTTCTGACCTTTGCCTCTT
TTATTATAGAGATGAGAAAGAAGAGGGTATCCTGGGAAGCATACTGTTAC
CTAGTTTTCAGATAGCTTTGCTTACCTCTGAAGATCACATTAATCGCAAA
TATGCTTTTAAGGCAGCCCATCCAAACATGCGGACCTATTATTTCTGCAC
TGATACAGGAAAGGAAATGGAGTTGTGGATGAAAGCCATGTTAGATGCTG
CCCTAGTACAGACAGAACCTGTGAAAAGAGTGGACAAGATTACATCTGAA
AATGCACCAACTAAAGAAACCAATAACATTCCCAACCATAGAGTGCTAAT
TAAACCAGAGATcCAAAACAATCAAAAAAACAAGGAAATGAGCAAAATTG
AAGAAAAAAAGGCATTAGAAGCTGAAAAATATGGATTTCAGAAgGATGGT
CAAGATAGACCCTTAACAAAAATTAATAGTGTAAAGCTGAATTCTCTGCC
ATCTGAATATGAGAGTGGGTCAGCATGCCCTGCTCAGACTGTGCACTACA
GACCAATCAACTTGAGCAGTTCAGAGAACAAAATAGTCAATGTTAGCCTG
GCAGATCTTAGAGGTGGAAATCGCCCCAATACAGGGCCCTTATACACAGA
GGCCGATCGAGTCATACAGAGAACAAATTCAATGCAGCAGTTGGAACAGT
GGATTAAAATCCAGAAGGGGAGGGGTCATGAAGAAGAAACCAGGGGAGTA
ATTTCTTACCAAACATTACCAAGAAATATGCCAAGTCACAGAGCCCAGAT
TATGGCCCGCTACCCTGAAGGTTATAGAACACTCCCAAGAAACAGCAAGA
CAAGGCCTGAAAGTATcTGCAGTGTAACCCCTTCCACTCATGACAAGACA
TTAGGACCCGGAGCGGAGGAGAAACGGAGGTCCATGAGAGATGACACAAT
GTGGCAGCTCTACGAATGGCAGCAGCGTCAGTTTTATAACAAACAGAGCA
CCCTCCCTCGACACAGTACTTTGAGTAGTCCCAAAACCATGGTAAATATT
TCTGACCAGACAATGCACTcTATTCCCACATCACCTTCCCACGGGTCAAT
AGCTGCTTATCAGGGATACTCCCCTCAACGAACTTACAGATCGGAAGTGT
cTTCACCAATTCAGAGAGGAGATGTGACAATAGACCGCAGACACAGGGCC
CATCACCCTAAGGTAAAATAGCTGCTGATTTTGTGTTAACTCACTACCTT
ATAAATGCTGTGTTTTCTTTCTAGTATACTATTTTAAATGTGAGAGACAA
AAGAATGGGGATAAAGTAAGCAAGGCAGCTCTTTTTTGTTTTAAAAAATA
AATAAAAATATTTTACAACAAAAAAAAAAAAAAAAAAA

(partial human PEPP2)

or

ATCAGAATGGCGGCGGATCTAAACCTGGAGTGGATCTCCCTGCCCCGGTC
CTGGACTTACGGGATCACCAGGGGCGGCCGAGTCTTCTTCATCAACGAGG
AGGCCAAGAGCACCACCTGGCTGCACCCCGTCACCGGCGAGGCGGTGGTC
ACCGGACACCGGCGGCAGAGCACAGATTTGCCTACTGGCTGGGAAGAAGC
ATATACTTTTAAAGGTGCAAGATACTATATAAACCACAATGAAAGGAAAG
TGACCTGCAAACATCCAGTCACAGGACAACCATCACAGGACAATTGTATT
TTTGTAGTGAATGAACAGACTGTTGCAACCATGACATCTGAAGAAAAGAA

GGAACGGCCAATAAGTATGATAAATGAAGCTTCTAACTATAACGTGACTT
CAGATTATGCAGTGCATCCAATGAGCCCTGTAGGCAGAACTTCACGAGCT
TCAAAAAAAGTTCATAATTTTGGAAAGAGGTCAAATTCAATTAAAAGGAA
TCCTAATGCACCGGTTGTCAGACGAGGTTGGCTTTATAAACAGGACAGTA
CTGGCATGAAATTGTGGAAGAAACGCTGGTTTGTGCTTTCTGACCTTTGC
CTCTTTTATTATAGAGATGAGAAAGAAGAGGGTATCCTGGGAAGCATACT
GTTACCTAGTTTTCAGATAGCTTTGCTTACCTCTGAAGATCACATTAATC
GCAAATATGCTTTTAAGGCAGCCCATCCAAACATGCGGACCTATTATTTC
TGCACTGATACAGGAAAGGAAATGGAGTTGTGGATGAAAGCCATGTTAGA
TGCTGCCCTAGTACAGACAGAACCTGTGAAAAGAGTGGACAAGATTACAT
CTGAAAATGCACCAACTAAAGAAACCAATAACATTCCCAACCATAGGGTG
CTAATTAAACCAGAGATCCAAAACAATCAAAAAAACAAGGAAATGAGCAA
AATTGAAGAAAAAAAGGCATTAGAAGCTGAAAAATATGGATTTCAGAAGG
ATGGTCAAGATAGACCCTTAACAAAAATTAATAGTGTAAAGCTGAATTCT
CTGCCATCTGAATATGAGAGTGGGTCAGCATGCCCTGCTCAGACTGTGCA
CTACAGACCAATCAACTTGAGCAGTTCAGAGAACAAAATAGTCAATGTTA
GCCTGGCAGATCTTAGAGGTGGAAATCGCCCCAATACAGGGCCCTTATAC
ACAGAGGCCGATCGAGTCATACAGAGAACAAATTCAATGCAGCAGTTGGA
ACAGTGGATTAAAATCCAGAAGGGGAGGGGTCATGAAGAAGAAACCAGGG
GAGTAATTTCTTACCAAACATTACCAAGAAATATGCCAAGTCACAGAGCC
CAGATTATGGCCCGCTACCCTGAAGGTTATAGAACACTCCCAAGAAACAG
CAAGACAAGGCCTGAAAGTATCTGCAGTGTAACCCCTTCCACTCATGACA
AGACATTAGGACCCGGAGCGGAGGAGAAACGGAGGTCCATGAGAGATGAC
ACAATGTGGCAGCTCTACGAATGGCAGCAGCGTCAGTTTTATAACAAACA
GAGCACCCTCCCTCGACACAGTACTTTGAGTAGTCCCAAAACCATGGTAA
ATATTTCTGACCAGACAATGCACTCTATTCCCACATCACCTTCCCACGGG
TCAATAGCTGCTTATCAGGGATACTCCCCTCAACGAACTTACAGATCGGA
AGTGTCTTCACCAATTCAGAGAGGAGATGTGACAATAGACCGCAGACACA
GGGCCCATCACCCTAAGCATGTCTATGTGCCTGACAGAAGGTCAGTGCCA
GCTGGCCTGACTTTACAGTCTGTTAGTCCCCAGAGCCTCCAAGGGAAAAC
GCTGTCACAAGATGAAGGTAGAGGCACATTATACAAATACAGACCTGAAG
AAGTAGATATTGATGCCAAGTTAAGCCGATTATGTGAACAAGATAAAGTG
GTGCATGCTCTGGAAGAGAAACTTCAGCAACTCCACAAGGAGAAATACAC
GCTTGAGCAAGCTTTGCTATCAGCCAGCCAAGAGATAGAAATGCATGCAG
ATAACCCAGCAGCCATTCAGACAGTGGTGTTACAAAGGGATGATTTACAA
AATGGACTGCTTAGTACGTGTCGAGAACTTTCTCGAGCCACTGCCGAATT
GGAACGAGCATGGAGAGAATATGATAAGTTAGAATACGATGTAACTGTTA
CCAGGAACCAGATGCAAGAGCAGCTGGATCACCTTGGTGAAGTTCAGACG
GAATCAGCAGGAATTCAGCGTGCACAGATTCAGAAAGAACTTTGGCGAAT
TCAGGATGTCATGGAAGGGCTGAGTAAACATAAGCAGCAAAGAGGTACTA
CAGAAATAGGTATGATAGGATCAAAGCCTTTCTCAACAGTTAAGTACAAA
AATGAGGGTCCAGATTATAGACTCTACAAGAGTGAACCAGAGTTAACAAC
AGTGGCAGAAGTTGATGAATCTAATGGAGAAGAAAAATCAGAACCTGTTT
CAGAGATAGAAACTTCAGTTGTTAAAGGTTCCCACTTTCCTGTTGGAGTA

GTCCCTCCAAGAGCAAAATCACCAACACCCGAATCTTCGACAATAGCTTC
CTATGTAACCTTGAGGAAAACTAAGAAGATGATGGATCTAAGAACGGAAA
GACCAAGAAGTGCAGTGGAACAGCTCTGTTTGGCTGAAAGTACTCGACCA
AGGATGACTGTGGAAGAGCAAATGGAAAGAATAAGAAGACATCAACAAGC
GTGCCTGAGGGAGAAGAAAAAAGGGTTAAATGTTATCGGTGCTTCAGACC
AGTCACCCTTACAAAGCCCTTCAAATTTAAGGGATAATCCATTTAGGACT
ACTCAGACTCGAAGGAGGGATGATAAGGAACTGGACACTGCCATTAGAGA
AAATGATGTAAAGCCAGACCATGAAACTCCTGCAACAGAAATTGTTCAAC
TAAAAGAAACCGAACCCCAAAATGTGGACTTCAGCAAAGAGTTAAAAAAA
ACTGAAAACATTTCATATGAAATGCTTTTTGAACCTGAGCCAAATGGAGT
AAATTCTGTGGAAATGATGGATAAAGAAAGAAACAAAGACAAAATGCCTG
AGGATGTTACATTCAGCCCTCAAGATGAAACACAGACCGCAAATCATAAA
CCAGAAGAGCATCCTGAAGAAAATACAAAGAACAGTGTTGACGAACAGGA
AGAAACTGTTATTTCTTACGAATCAACTCCTGAGGTTTCTAGAGGAAATC
AAACAATGGCAGTGAAAAGTCTGTCCCCATCTCCTGAGTCCTCGGCATCG
CCAGTTCCATCCACTCAGCCGCAGCTCACAGAAGGATCACATTTCATGTG
TGTGTAGTCTTAGAAGAACTATACTGACTTCTGTTGAAACCATTCAAAGC
TAAAGACATGGACCTTCAGCAGTGTAAGAAGATATTGTACAGTATATTTT
AAATCTATGAAATTCATAGTTCTGATGCTTTTGGTCACAGAGCATCATTT
TATCACTTCTGGAAAATGTTTATTCCAAAACAGCTTTAATGGCCCATATG
TACACTTCGTAATCTCAAGGTTATTATTCTGACACCAGCTTGCTGCTATG
ATTTCAGAGCACATAAGTAAAGGTGCTTTTTAATGTGCAGTCTATTTCCA
GAGCTTACTTAGTTGCTGATTTCCAGATTTCGATGTTTCTTAAGTCTAGG
TGAATTTATATATATATTTTTTTGCTTTTCATTTTCTAAAGTTAGTTATT
ATTTCCATTGAAGCTTGTTTTCTTTTTTTCTTCCCATTTTAGCTACTGCA
GTGCTTTTGTTTCACACTTGATTTGTAAAAATTTTATATATATGTATTTA
AAATGTGCCATTTTATTGCTAAGTGAAGTATGTCCTGTTTTCTGCTATAA
TTCTTTCTCGGTCAGATTGCAATGTCAGCAGTTACTGCCACACTCCTGTC
AGCTTAAACACAAATGTTACTGCTTATCTTTTCTTAAAAAAAAAAAAAAC
AAAGTGTAGGTATTTTGAAGTACTGGGCTTATATTTCATTGGAATACATG
TGTACAGCAATAAGCAGGTTTCCAAATCCGGTACTTAGTTTGTGTACAAA
TGTAATTATGTTCATTGTGTATATATTATACAATGAGCACATGTAATGTA
TTAAAGGCTACTTACTATTGTTTAAATGCAAATGTTCATATCTCATTTCT
TTTTTTATCATGTTAAATAAATGTTGATGTTCTTAAAAAAAAAAAAAAAA
AAA

(human PEPP2)

or

atgtccaataaaacaggtgggaaacgcccggctaccaccaacagtgacat
acccaaccacaacatggtgtccgaggtccctccagagcggcccagcgtcc
gggcaactcgcacagcccgcaaagccatcgcctttggcaagcgctcacac
tccatgaagcggaaccccaatgcacctgtcaccaaggcgggctggctctt

caaacaggccagctccggggttaagcagtggaacaagcgctggttcgtcc
tggtggatcgctgcctcttctactataaagatgagaaggaagagagtatc
ctgggcagcatccccctcctgagcttccgggtagccgcagtgcagccctc
agacaacatcagccggaaacacacgtttaaggctgagcatgccggggtcc
gcacctacttcttcagtgccgagagccccgaggagcaagaggcctggatc
caggccatgggggaggctgctcgagtacagatccctccagcccagaagtc
agtgccccaagctgtgcggcacagccatgagaagccagactcggagaacg
tcccacccagcaagcaccaccagcagccaccccacaacagcctccctaag
cctgagccagaggccaagactcgaggggagggtgatggccgaggctgtga
gaaggcagagagaaggcctgagaggccagaagtcaagaaagagcctccgg
tgaaagccaatggcctcccagctggaccggagccagcctcagagccgggc
agcccttaccccgagggcccaagagtgccaggggtggggaacagcctgc
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ggacagcctccacacctacaagttaaacgagcaagacacagataagctgc
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gacccaccaggagctggagatgtttggaagccagcccgcctacccagaaa
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tgagcacctcgagtctgaggtctctgccctgcacgatgacctctgggagc
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gagatctggaggatccaggacgtgatggaggggctgaggaagaacaaccc
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ccacctacagctccaacagcccggccagccccctcagctctgccagcctc
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aagacccccaccagacattgcccctggacacccccagagacatcagcctt
gtgcccaccaggcaagaggtagaggcagagaagcaggcagctctcaacaa
agttggcgttgtgcccccctcggacaaaatcgcccactgatgatgaggtga
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tcctcccaggaacgccccaagagtgctgtgtttcctggcgaggggaaggt
caagatgagcgtggaggagcagattgaccgaatgcggcggcaccagagtg
gctccatgaaggagaagcggaggagcctgcagctcccggccagcccggcc
cccgaccccagtccccggccagcctacaaagtggtgcgccgccaccgcag
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agcctggcgggcatgcctacgagacaccccgggaggaaattgcccggctt
cgcaaaatggagctagagccccagcattatgacgtggacatcaataagga
gctctccactccagacaaagtcctcatccctgaacggtacattgacctgg
agcctgacactcccctgagccctgaggagttgaaggagaagcagaagaag
gtggagaggatcaagacactcattgccaaatccagtatgcagaacgtggt
gcccatcggcgaggggactctgtggacgtgccccaggactcagagagcc
agctgcaggagcaggagaagcggattgaaatctcctgcgccctggcgacc
gaggcctcccgcaggggccgcatgctgtctgtgcaatgtgccaccccaag
ccctcccacctcccctgcttccccggctcctccagcaaaccccctgtcgt
ctgaatccccacggggcgccgacagcagctataccatgcgggtctga

(human PEPP3)

or

ACGAGGCTTACCGGGAATGTCTGGGCCCGCGCCTCGCGGCCCCCAAGCTC
CACGCTGCGCCCGCTGTCCCGGCCTCTAAAGGCCGCCACGTCCCTGCGGC
GCGCGCAGGCAGAAAGCGGCTTCGTGCCGGCGGAGGGGGCCCGGGCGGGC
CGGGAGGGGCTGCCCCAGGCCCTGCGCCTACCCCATCACCGCGGCCGGCG
CCGGGCCGGGAGGATGCGCGGTGTGGGGCTCTGAAGCATGGAGGGGGTGT
TGTACAAGTGGACCAACTATCTCACAGGCTGGCAGCCTCGTTGGTTTGTT
TTAGATAATGGAATCTTATCCTACTATGATTCACAAGATGATGTTTGCAA
AGGGAGCAAAGGAAGCATAAAGATGGCAGTTTGTGAAATTAAAGTTCATT
CAGCAGACAACACAAGAATGGAATTAATCATTCCTGGAGAGCAGCATTTC
TACATgAAGGCAGTGAATGCAGCTgAAAgACAgAgGTGGCTGGTCGCTCT
GGGGAGCTCCAAAGCATGTTTGACTGATACAAGGACTAAAAAAGAAAAAG
AAATAAGTGAAACCAGTGAATCGCTGAAAACCAAAATGTCTGAACTTCGC
CTCTACTGTGACCTCTTAATGCAGCAAGTTCATACAATACAGGAATTTGT
TCACCATGATGAGAATCATTCATCTCCTAGTGCAGAGAACATGAATGAAG
CCTCTTCTCTGCTTAGTGCCACGTGTAATACATTCATCACAACGCTTGAG
GAATGTGTGAAGATAGCCAATGCCAAGTTTAAACCTGAGATGTTTCAACT
GCACCATCCGGATCCCTTAGTTTCTCCTGTGTCACCTTCTCCTGTTCAAA
TGATGAAGCGTTCTGTCAGCCACCCTGGTTCTTGCAGTTCAGAGAGGAGT
AGCCACTCTATAAAAGAACCAGTATCTACACTTCACCGACTCTCCCAGCG
ACGCCGAAGAACCTACTCAGATACAGATTCTTGTAGTGATATTCCTCTTG
AAGACCCAGATAGACCTGTTCACTGTTCAAAAAATACACTTAATGGAGAT
TTGGCATCAGCAACCATTCCTGAAGAAAGCAGACTTACGGCCAAAAAACA

ATCTGAATCAGAAGATACTCTTCCATCCTTCTCTTCCTGAAGAAACTGAA
GTGTCCAACTTCCTCTAAGTATTGCTATGCAAAAGCTGCTGTAATTAAAC
TATTGTTATAGGGAGTAGTTTTTTCCCTTAGGACTCTGCACTTTATAGAA
TGTTGTAAAACAGACAAACAAGAAAACAAACCACATACTTTTGAAGTGTA
TTTTATCTTTATATAGTTTGTTTGCAAGAGTATTTTCCTAATAACTTCAC
AGTATGAATGTGCATCTTTTTTTTTTGAACAAATGATGGTGTAACATTTT
GACATCCATAAGGACAAATGTAGATATTTTTCTTAAAAACTCTGAGGGGA
CTGACAGCATGGTCAGGGTGTATTGTAGCTTATAAACATGAAATCTTaTT
AGGGTTTCCGTTTGACAGAAGTGTGATATATGTaACTTGTGCCATGGACC
AAATGGTCACTTTACCACAGCTAAAATGAGTTaCGATAGCAGCTTGATGG
TGATgGTaTGTATTCCTTTAATCAAAAAGGAACaCAATATTcTAAGTATC
TTTAGCCCAATACCATGACATATTGaGCATCTTTAAATAACCaGaCTGTA
TTGTCCTTCAtAATGtGAAGTTGACACTACTGATTTGTCAAtACCAAATT
TTGGGTTAAAGTGTTTAATTTTTATGTATTTATTTTCTTGTTGCCTCAAA
AGATGATTGCATTCTAACTTTTGTGACCTACCAAATTTAAGATGGGTATA
CGTTGTTCTTTACGTTGTTCTAGAAAAGAGATTTTAATGCTGTAGTGACT
TTGCTCACTTACACTAGAGAAATAAACAACTTTCAATGGAAGAGAATTTT
AGTGCTTTTTTTTTCCTAAAATAGATATTAAGCTGCTGTTGTAAAGTATT
GTTTGCAGCTCTTTCCAATATCTAGAGACATTTTTATTTATGAATATTTA
TACcAAAAGGAATTCTGTCAAGATGACTGCTcTATATCACTTGAGAATGG
CATTATTTAATTAAAGAACAAATAGCATTTTTTGGTAGTGCCTGTCCATA
CCTATTGTCATTGTTTGCCTTGTAATCTGTTTTTTTGAATTCATTTTGGG
CTGATAGTTTTGTTTAAGGTTTTGGATAAGGAGCACTTTAAAACAAACTG
GTGTGTTGTTTTTAAGTTAATCATATGTTTAATAAATGCGTGGTTTTTGC
ATTCAAACACATCcAAAAAAAAAaAAAAGGAA*AGGA*GAAAAAAAAAA

(human FAPP1)

or

ctgcgggcccgcgcctccgcagcagcgcgccggcgcgggccaggaggatg
cgcgcgccggctctgaagcatggaggggttctgtacaagtggaccaact
atctcacaggttggcagcctcgatggtttgttctggataatggaatcctg
tcctactatgactcacaggatgatgtctgcaaagggagcaaagggagtat
aaagatggcggtctgtgagattaaagtccatcccgcagacaacacaagaa
tggagttaatcattccaggagagcagcatttctacatgaaggcagtaaat
gccgccgagagacagaggtggctggttgcccttgggagctccaaagcgtg
tttgaccgacacgaggactgcaaaagagaaagaaataagtgagaccagtg
aatctctgaaaaccaaaatgtctgaacttcgcctctactgtgacctcctg
atgcagcaggttcatacgatccaggaattcgtccaccgtgatgagaggca
tccctctcccagtgtggagaacatgaatgaagcctcctccttgctcagtg
ccacctgtaacacattcatcacaaccctggaggagtgtgtgaagatcgcc
aacgccaagtttaaacctgagatgtttcaactgcctcatccggatcccct

ggtctctcccgtgtcgccttctcctgttcagatgatgaagcgttcagcca
gccaccctggttcctgcagttccgagaggagcagctgctccatcaaagaa
ccagcatctgccctccaccgacttcctcagcgacgccgcagaacctactc
ggacacagactcttgtaatgatgttccccctgaagacccagagagacctc
ttcactgttcaggaaacacacttaatggagatttggcatcagcaaccatt
ccggaagaaagcagactcatggccaagacacaatctgaagaacctcttct
gcccttctcctgaggaaacagacatgcccagcttcctcctgaggaaacag
acatgcccagcttcctcctgaggaaacagacatgcccagcttcctcctga
ggaaacagacatgcccagcttcctctgagtgtcgctatgcaaaagctgct
gtaattaaactcggtctgggctagctttgccctctccttaggatttctct
gcactttatagaatattgtaaacaaacaacccacatacttttgaagtgta
ttttatctttctatagtttacttgcaagagtattttcctaataacttcac
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tttgacatccataaggacaaatgtagatatttttctaaaaaactgtgagg
gactgacagcttggtcagtgtgtattgtagtatataaacatgaaatctcg
ccagatttatttgacagaaatgtgagagatgtaacttgtgccatggacca
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tgattatatcatattcctttaagcaaaaaggaaacgcttaatattctaaa
ggtctttagcccaaataccatgacatattgagcatttttttttaaaaagc
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gcatcagaagatggttatgtcctaacttttatggcctccccaatttaaga
tgtatatgcatagttgttattacgttgttctaagatacatgaggcaagtg
tcccagtgatcttgttcccttacacgagagaagtaaacagctttcaatgg
gaatggagttcagtgcttttcagaaaataggcagcaagctgctgttgtaa
ggtatgatttgcagctctttggcatatctagagacatttttaatttatga
atatttatacaaaaagcaattctgtcaagatgactgttctatatcacttg
agaatggcattatttaattaaagaacaatttgcagtt

(mouse FAPP1)

or

GGTGCTCCTCGCCTCTTGGGGCCTGGGGCAGTGAGGGGGCCGGCGGGCGT
GGGCCGAGTGGCCGCGGGCGCCATGGAGGGGGTGCTGTACAAGTGGACCA
ACTATCTGAGCGGTTGGCAGCCTCGATGGTTCCTTCTCTGTGGGGGAATA
TTGTCCTATTATGATTCTCCTGAAGATGCCTGGAAAGGTTGCAAAGGGAG
CATACAAATGGCAGTCTGTGAAATTCAAGTTCATTCTGTAGATAATACAC
GCATGGACCTGATAATCCCTGGGGAACAGTATTTCTACCTGAAGGCCAGA
AGTGTGGCTGAAAGACAGCGGTGGCTGGTGGCCCTGGGATCAGCCAAGGC
TTGCCTGACTGACAGTAGGACCCAGAAGGAGAAAGAGTTTGCTGAAAACA
CTGAAAACTTGAAAACCAAAATGTCAGAACTAAGACTCTACTGTGACCTC
CTTGTTCAGCAAGTAGATAAAACAAAAGAAGTGACCACAACTGGTGTGTC
CAATTCTGAGGAGGGAATTGATGTGGGAACTTTGCTGAAATCAACCTGTA

ATACTTTTCTGAAGACCTTGGAAGAATGCATGCAGATTGCAAATGCAGCC
TTCACCTCTGAGCTGCTCTACCACACTCCACCAGGATCACCACAGCTGGC
CATGCTCAAGTCCAGCAAGATGAAACATCCTATTATACCAATTCATAATT
CATTGGAAAGGCAAACGGAGTTGAGCACTTGTGAAAATGGATCTTTAAAT
ATGGAAATAAATGGTGAGGAAGAAATCCTAATGAAAAATAAGAATTCCTT
ATATTTGAAATCTGCAGAGATAGACTGCAGCATATCAAGTGAGGAAAATA
CAGATGATAATATAACCGTCCAAGGTGAAATAATGAAGGAAGATAGAATG
GAAAACCTGAAAAATCATGACAATAACTTGTCTCAGTCTGGATCAGACTC
AAGTTGCTCTCCAGAATGCCTCTGGGAGGAAGGCAAAGAAGTTATCCCAA
CTTTCTTTAGTACCATGAACACAAGCTTTAGTGACATTGAACTTCTGGAA
GACAGTGGCATTCCCACAGAAGCATTCTTGGCATCATGTTGTGCTGTGGT
TCCAGTATTAGACAAACTTGGCCCTACAGTGTTTGCTCCTGTTAAGATGG
ATCTTGTTGAAAATATTAAGAAAGTAAATCAGAAGTATATAACCAATAAA
GAAGAGTTTACCACTCTCCAGAAGATAGTGCTGCACGAAGTGGAGGCGGA
TGTAGCCCAGGTTAGGAACTCAGCGACTGAAGCCCTCTTGTGGCTGAAGA
GAGGTCTCAAATTTTTGAAGGGATTTTTGACAGAAGTGAAAAATGGGGAA
AAGGATATCCAGACAGCCCTGAATAACGCATATGGTAAAACATTGCGGCA
ACACCATGGCTGGGTAGTTCGAGGGGTTTTTGCGTTAGCTTTAAGGGCAA
CTCCATCCTATGAAGATTTTGTGGCCGCGTTAACCGTAAAGGAAGGTGAC
CACCGGAAAGAAGCTTTCAGTATTGGGATGCAGAGGGACCTCAGCCTTTA
CCTCCCTGCCATGAAGAAGCAGATGGCCATACTGGACGCTTTATAAGAGG
TCCATGGGCTGGAATCTGATGAGGTTGTATGATGGCTGCTGGGCAGCACC
TCCTAACTTCAGGGAATAAAGTGCTAAAGTGTTTTGTTGCCCTACTTAAT
TTCCAGCAACAGCCTCAACCCTCTCCAACCCCTTCACCTGGGGGGATGGA
CAGGAGGTGGCAAAACCCAGTGCTTTTATAATTTTTAAAATGCATATGTG
TTTTGTTTAAAGATCAAGGTGCTATATATTTCAGTTCAGCAGGCCTACTG
GAAACCAAATGATAAGCTGCTGTAGACTTGAACAGCAAGTTATAAGAGCA
GATTTAACAAACAAA

(human FAPP2)

or a variant, fragment, fusion or derivative thereof.

References for full length sequences of centaurin-β2 and AtPH1 are given in Example 1, for example in Table 1. Polynucleotides encoding full-length centaurin-β2 or AtPH1 are excluded from the polynucleotides of the invention.

It will be appreciated that sequences encoding other full length TAPP, PEPP and FAPP polypeptides, for example other mammalian TAPP polypeptides,

may be obtained by routine use of methods well known to those skilled in the art, making use of the sequences shown above. Thus PCR methods may be used, particularly methods developed to generate 5' cDNA sequences (for example, the "RACE" method, as well known to those skilled in the art). Such methods may be used in conjunction with sequence database analysis, for example EST database analysis and sequencing, as well known to those skilled in the art.

It will be appreciated that an expressed sequence tag (EST) clone is not a recombinant polynucleotide as defined above as it lacks sequences necessary for the translation and therefore expression of the expressed sequence tag. EST sequences may be cloned in the vector Uni-ZAP XR, pT7T3D-Pac, pBluescript SK-, Lafmid BA or pCMV-SPORT2 vector.

A polynucleotide comprising a fragment of the recombinant polynucleotide encoding a polypeptide of the invention or a variant, fragment, fusion or derivative may also be useful. Preferably, the polynucleotide comprises a fragment which is at least 10 nucleotides in length, more preferably at least 14 nucleotides in length and still more preferably at least 18 nucleotides in length. Such polynucleotides are useful as PCR primers. A polynucleotide complementary to the polynucleotide (or a fragment thereof) encoding a polypeptide of the invention or a variant, fragment, fusion or derivative may also be useful. Such complementary polynucleotides are well known to those skilled in the art as antisense polynucleotides.

The polynucleotide or recombinant polynucleotide of the invention may be DNA or RNA, preferably DNA. The polynucleotide may or may not contain introns in the coding sequence; preferably the polynucleotide is a cDNA.

A "variation" of the polynucleotide includes one which is (i) usable to produce a protein or a fragment thereof which is in turn usable, for example a processed polypeptide as described above, or to prepare antibodies which specifically bind to the protein encoded by the said polynucleotide or (ii) an antisense sequence corresponding to the gene or to a variation of type (i) as just defined. For example, different codons can be substituted which code for the same amino acid(s) as the original codons. Alternatively, the substitute codons may code for a different amino acid that will not affect the activity or immunogenicity of the protein or which may improve or otherwise modulate its activity or immunogenicity. For example, site-directed mutagenesis or other techniques can be employed to create single or multiple mutations, such as replacements, insertions, deletions, and transpositions, as described in Botstein and Shortle, "Strategies and Applications of In Vitro Mutagenesis" *Science*, **229**: 193-210 (1985), which is incorporated herein by reference. Since such modified polynucleotides can be obtained by the application of known techniques to the teachings contained herein, such modified polynucleotides are within the scope of the claimed invention.

Moreover, it will be recognised by those skilled in the art that the polynucleotide sequence (or fragments thereof) encoding a polypeptide of the invention can be used to obtain other polynucleotide sequences that hybridise with it under conditions of high stringency. Such polynucleotides includes any genomic DNA. Accordingly, the polynucleotide of the invention includes polynucleotide that shows at least 80%, preferably 85%, and more preferably at least 90% and most preferably at least 95% homology with the polynucleotide identified in the method of the invention, provided that such homologous polynucleotide encodes a polypeptide which

is usable in at least some of the methods described below or is otherwise useful. Moreover, it will be recognised by those skilled in the art that the polynucleotide sequence (or fragments thereof) encoding a polypeptide of the invention can be used to obtain other polynucleotide sequences that hybridise with it under conditions of high stringency. Such polynucleotides includes any genomic DNA. Accordingly, the polynucleotide of the invention includes polynucleotide that shows at least 60%, preferably 70%, and more preferably at least 80% and most preferably at least 90% homology with the polynucleotide identified in the method of the invention, provided that such homologous polynucleotide encodes a polypeptide which is usable in at least some of the methods described below or is otherwise useful. As noted above, a polynucleotide encoding full length centaurin-β2 or AtPH1 is not a polynucleotide of the invention.

Per cent homology can be determined by, for example, the GAP program of the University of Wisconsin Genetic Computer Group.

DNA-DNA, DNA-RNA and RNA-RNA hybridisation may be performed in aqueous solution containing between 0.1XSSC and 6XSSC and at temperatures of between 55°C and 70°C. It is well known in the art that the higher the temperature or the lower the SSC concentration the more stringent the hybridisation conditions. By "high stringency" we mean 2XSSC and 65°C. 1XSSC is 0.15M NaCl/0.015M sodium citrate. Polynucleotides which hybridise at high stringency are included within the scope of the claimed invention.

"Variations" of the polynucleotide also include polynucleotide in which relatively short stretches (for example 20 to 50 nucleotides) have a high degree of homology (at least 80% and preferably at least 90 or 95%) with

equivalent stretches of the polynucleotide of the invention even though the overall homology between the two polynucleotides may be much less. This is because important active or binding sites may be shared even when the general architecture of the protein is different.

A variety of methods have been developed to operably link polynucleotides, especially DNA, to vectors for example via complementary cohesive termini. Suitable methods are described in Sambrook *et al*(1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

A desirable way to modify the DNA encoding a polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* **239**, 487-491. This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art.

In this method the DNA to be enzymatically amplified is flanked by two specific primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

The DNA (or in the case of retroviral vectors, RNA) is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the DNA encoding the polypeptide constituting the compound of the invention may be used in accordance with known techniques, appropriately modified in view of the teachings contained

herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et al*and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide constituting the compound of the invention may be joined to a wide variety of other DNA sequences for introduction into an appropriate host. The companion DNA will depend upon the nature of the host, the manner of the introduction of the DNA into the host, and whether episomal maintenance or integration is desired.

Generally, the DNA is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a DNA sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the

gene for such selectable trait can be on another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the recombinant DNA of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*), filamentous fungi (for example Aspergillus), plant cells, animal cells and insect cells.

The vectors include a prokaryotic replicon, such as the ColE1 ori, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical prokaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and pTrc99A and pKK223-3 available from Pharmacia, Piscataway, NJ, USA.

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

As described in Example 1, the pEBG-2T expression vector may be used to express GST fusion proteins in eukaryotic cells, for example in 293 cells (human embryonic kidney cells).

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, TRP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers

HIS3, TRP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Yeast host cells include YPH499, YPH500 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Preferred mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658, and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650. Preferred insect cells are Sf9 cells which can be transfected with baculovirus expression vectors.

Transformation of appropriate cell hosts with a DNA construct of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen *et al*(1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110 and Sambrook *et al*(1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Transformation of yeast cells is described in Sherman *et al*(1986) Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor,

NY. The method of Beggs (1978) *Nature* **275**, 104-109 is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA.

Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cell, bacterial cells, insect cells and vertebrate cells.

For example, many bacterial species may be transformed by the methods described in Luchansky *et al*(1988) *Mol. Microbiol.* **2**, 637-646 incorporated herein by reference. The greatest number of transformants is consistently recovered following electroporation of the DNA-cell mixture suspended in 2.5X PEB using 6250V per cm at 25:FD.

Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* **194**, 182.

Successfully transformed cells, ie cells that contain a DNA construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* **98**, 503 or Berent *et al*(1985) *Biotech.* **3**, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant DNA, successful transformation can be confirmed by well known immunological methods when the recombinant DNA is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium.

A further aspect of the invention provides a method of making the polypeptide of the invention or a variant, derivative, fragment or fusion thereof or a fusion of a variant, fragment or derivative the method comprising culturing a host cell comprising a recombinant polynucleotide or a replicable vector which encodes said polypeptide, and isolating said polypeptide or a variant, derivative, fragment or fusion thereof or a fusion of a variant, fragment or derivative from said host cell. Methods of cultivating host cells and isolating recombinant proteins are well known in the art.

The invention also includes a polypeptide, or a variant, fragment, derivative or fusion thereof, or fusion of a said variant or fragment or derivative obtainable by the above method of the invention.

A still further aspect of the invention provides an antibody reactive towards a polypeptide of the invention, for example TAPP, PEPP or FAPP, or a

fragment thereof. It is preferred that the antibody is not an antibody reactive towards centaurin-β2 or AtPH1.

It is preferred that the antibody does not react substantially with another polypeptide comprising a PH domain. Accordingly, it may be preferred if peptides based on the TAPP, PEPP or FAPP sequence are used which vary significantly from any peptides found in any other PH domains, for example in the polypeptides indicated in part A of Table 1.

Antibodies reactive towards the said polypeptide of the invention may be made by methods well known in the art. In particular, the antibodies may be polyclonal or monoclonal.

Suitable monoclonal antibodies which are reactive towards the said polypeptide may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", SGR Hurrell (CRC Press, 1982).

In a preferred embodiment the antibody is raised using any suitable peptide sequence obtainable from the given amino acid sequence, for example of TAPP, PEPP or FAPP. It is preferred if polyclonal antipeptide antibodies are made. In a preferred embodiment of the invention, an antibody of the invention is capable of preventing or disrupting the interaction between a polypeptide of the invention or a fragment thereof and an interacting polypeptide identified by the method of the invention described above, or a phosphoinositide. Such antibodies are believed to be useful in medicine, for example in treating cancer or promoting apoptosis.

Peptides in which one or more of the amino acid residues are chemically modified, before or after the peptide is synthesised, may be used providing that the function of the peptide, namely the production of specific antibodies *in vivo*, remains substantially unchanged. Such modifications include forming salts with acids or bases, especially physiologically acceptable organic or inorganic acids and bases, forming an ester or amide of a terminal carboxyl group, and attaching amino acid protecting groups such as N-t-butoxycarbonyl. Such modifications may protect the peptide from *in vivo* metabolism. The peptides may be present as single copies or as multiples, for example tandem repeats. Such tandem or multiple repeats may be sufficiently antigenic themselves to obviate the use of a carrier. It may be advantageous for the peptide to be formed as a loop, with the N-terminal and C-terminal ends joined together, or to add one or more Cys residues to an end to increase antigenicity and/or to allow disulphide bonds to be formed. If the peptide is covalently linked to a carrier, preferably a polypeptide, then the arrangement is preferably such that the peptide of the invention forms a loop.

According to current immunological theories, a carrier function should be present in any immunogenic formulation in order to stimulate, or enhance stimulation of, the immune system. It is thought that the best carriers embody (or, together with the antigen, create) a T-cell epitope. The peptides may be associated, for example by cross-linking, with a separate carrier, such as serum albumins, myoglobins, bacterial toxoids and keyhole limpet haemocyanin. More recently developed carriers which induce T-cell help in the immune response include the hepatitis-B core antigen (also called the nucleocapsid protein), presumed T-cell epitopes such as Thr-Ala-Ser-Gly-Val-Ala-Glu-Thr-Thr-Asn-Cys, beta-galactosidase and the 163-171 peptide of interleukin-1. The latter compound may variously be regarded as

a carrier or as an adjuvant or as both. Alternatively, several copies of the same or different peptides of the invention may be cross-linked to one another; in this situation there is no separate carrier as such, but a carrier function may be provided by such cross-linking. Suitable cross-linking agents include those listed as such in the Sigma and Pierce catalogues, for example glutaraldehyde, carbodiimide and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate, the latter agent exploiting the -SH group on the C-terminal cysteine residue (if present).

If the peptide is prepared by expression of a suitable nucleotide sequence in a suitable host, then it may be advantageous to express the peptide as a fusion product with a peptide sequence which acts as a carrier. Kabigen's "Ecosec" system is an example of such an arrangement.

The peptide of the invention may be linked to other antigens to provide a dual effect.

It will be appreciated that other antibody-like molecules may be useful in the practice of the invention including, for example, antibody fragments or derivatives which retain their antigen-binding sites, synthetic antibody-like molecules such as single-chain Fv fragments (ScFv) and domain antibodies (dAbs), and other molecules with antibody-like antigen binding motifs. Such antibody-like molecules are included by the term antibody as used herein.

It will be appreciated that peptidomimetic compounds may also be useful in the practice of the invention. Thus, by "polypeptide" or "peptide" we include not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is

reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Mézière *et al* (1997) *J. Immunol.* 159, 3230-3237, incorporated herein by reference. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Mézière *et al* (1997) show that, at least for MHC class II and T helper cell responses, these pseudopeptides are useful. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis.

Similarly, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the Cα atoms of the amino acid residues is used; it is particularly preferred if the linker moiety has substantially the same charge distribution and substantially the same planarity as a peptide bond.

It will be appreciated that the peptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

A further aspect of the invention provides a polypeptide of the invention, or a fragment, fusion, variant or derivative thereof, or fusion of a fragment, variant or derivative, for example TAPP, PEPP or FAPP or a fragment thereof, for use in medicine. Preferences for the said variant, fragment, derivative or fusion or a fusion of a variant, fragment or derivative are as indicated above.

A further aspect of the invention provides a nucleic acid of the invention for use in medicine.

A further aspect of the invention provides a compound of the invention or other compound identifiable by or identified in a screening assay of the invention or an antibody of the invention for use in medicine.

Conditions or diseases in which the polypeptides, polynucleotides, compounds or antibodies of the invention may be particularly useful are indicated above.

A further aspect of the invention provides an interacting polypeptide of the invention or nucleic acid of the invention or antibody of the invention for use in medicine. A still further aspect of the invention provides a pharmaceutical composition comprising a polypeptide (including fragments, variants, derivatives and fuions), interacting polypeptide, nucleic acid, antibody and/or compound of the invention and a pharmaceutically acceptable carrier. A suitable carrier will be known to those skilled in the art.

The polypeptide, interacting polypeptide, polynucleotide, compound, antibody, composition or medicament of the invention may be administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or intravesically, in standard sterile, non-pyrogenic formulations of diluents and carriers. The polypeptide, interacting polypeptide, polynucleotide, compound, antibody, composition or medicament of the invention may also be administered in a localised manner, for example by injection. In general, the compound is administered orally, although this is not preferred for peptides. The compound may be administered intravenously, parenterally or subcutaneously, although these are not preferred.

A derivative or fusion of a polypeptide of the invention or variant, fragment or fusion thereof which may be particularly useful, for example in medicine, may comprise the polypeptide of the invention or variant, fragment or fusion therof and a further portion. It is preferred that the said further portion confers a desirable feature on the said molecule; for example, the portion may useful in detecting or isolating the molecule, or promoting cellular uptake of the molecule or the interacting polypeptide. The portion may be, for example, a radioactive moiety, a fluorescent moiety, for example a small fluorophore or a green fluorescent protein (GFP) fluorophore, as well known to those skilled in the art. The moiety may be an immunogenic tag, for example a Myc, FLAG or HA (haemagglutinin) tag, as known to those skilled in the art or may be a lipophilic molecule or polypeptide domain that is capable of promoting cellular uptake of the molecule or the interacting polypeptide, as known to those skilled in the art, for example as characterised for a *Drosophila* polypeptide (see, for example, Derossi *et al* (1998) *Trends Cell Biol* **8**, 84-87). Further useful tags include a tag that is capable of being phosphorylated, for example a tag capable of being phosphorylated by protein kinase A. Such a tag may be useful in introducing a radioactive label, for example ^{32}P or ^{33}P, onto the polypeptide.

Compounds, identifiable in the screening method, which mimic the effect of a particular phosphoinositide on a polypeptide, for example TAPP, PEPP or FAPP, are believed to be useful in treating diabetes and/or other conditions, as indicated above. Compounds identifiable in the screening methods of the invention that inhibit binding of a phosphoinositide to the said polypeptide are believed to be useful in treating cancer. Compounds may be used, for example, for treatment of diabetes by switching on insulin-stimulated signal

transduction pathways or for the treatment of cancer by inhibiting cell proliferation or promoting apoptosis. Compounds may also be useful in the modulation or resolution of inflammation or platelet activation, as discussed above.

It will be appreciated that certain compounds found in the screening methods may be able to enhance cell proliferation in a beneficial way and may be useful, for example in the regeneration of nerves or in wound healing.

Thus, a further aspect of the invention provides a method of treating a patient in need of modulation of the activity of a said polypeptide of the invention, for example TAPP, PEPP or FAPP or with an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition), the method comprising administering to the patient an effective amount of a compound of the invention or a polypeptide of the invention or a variant, fragment, fusion or derivative or a fusion of a variant, fragment or derivative. By inflammatory disease is included immune system disorders, for example autoimmune diseases, as will be apparent to those skilled in the art.

A further aspect of the invention provides the use of a compound of the invention or a polypeptide of the invention or a variant, fragment, fusion or derivative or a fusion of a variant, fragment or derivative in the manufacture of a medicament for treatment of a patient in need of modulation of the activity of a polypeptide of the invention, for example TAPP, PEPP or FAPP, or with an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition).

A further aspect of the invention provides a compound capable of altering the expression of a polypeptide of the invention, for example TAPP, PEPP or FAPP. The said compound may be an antisense molecule or ribozyme directed (for example, capable of binding to a polynucleotide encoding TAPP, PEPP or FAPP under physiological conditions) against a polynucleotide encoding a polypeptide of the invention, for example TAPP, PEPP or FAPP. A further aspect of the invention provides a compound capable of altering the expression of a polypeptide of the invention, for example TAPP, PEPP or FAPP, for use in medicine. A still further aspect of the invention provides the use of a compound capable of altering the expression of a polypeptide of the invention, for example TAPP, PEPP or FAPP in the manufacture of a medicament for the treatment of a patient in need of modulation of the activity of a polypeptide of the invention, for example TAPP, PEPP or FAPP or with an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition).

It will be appreciated that the nucleic acid of the invention may be an antisense oligonucleotide, for example an antisense oligonucleotide directed against a nucleic acid encoding a polypeptide of the invention such as the human TAPP, PEPP or FAPP gene. Antisense oligonucleotides are single-stranded nucleic acid, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex is formed. These nucleic acids are often termed "antisense" because they are complementary to the sense or coding strand of the gene. Recently, formation of a triple helix has proven possible where the oligonucleotide is bound to a DNA duplex. It was found that oligonucleotides could recognise sequences in the major

groove of the DNA double helix. A triple helix was formed thereby. This suggests that it is possible to synthesise a sequence-specific molecules which specifically bind double-stranded DNA *via* recognition of major groove hydrogen binding sites.

The nucleic acid of the invention may be an antisense oligonucleotide, for example an antisense oligonucleotide directed against a nucleic acid encoding a polypeptide of the invention such as the human TAPP, PEPP or FAPP gene or an interacting polypeptide of the invention, which may be a receptor molecule. Antisense oligonucleotides are single-stranded nucleic acid, which can specifically bind to a complementary nucleic acid sequence. By binding to the appropriate target sequence, an RNA-RNA, a DNA-DNA, or RNA-DNA duplex is formed. These nucleic acids are often termed "antisense" because they are complementary to the sense or coding strand of the gene. Recently, formation of a triple helix has proven possible where the oligonucleotide is bound to a DNA duplex. It was found that oligonucleotides could recognise sequences in the major groove of the DNA double helix. A triple helix was formed thereby. This suggests that it is possible to synthesise a sequence-specific molecules which specifically bind double-stranded DNA *via* recognition of major groove hydrogen binding sites.

By binding to the target nucleic acid, the above oligonucleotides can inhibit the function of the target nucleic acid. This could, for example, be a result of blocking the transcription, processing, poly(A)addition, replication, translation, or promoting inhibitory mechanisms of the cells, such as promoting RNA degradations.

Antisense oligonucleotides are prepared in the laboratory and then introduced into cells, for example by microinjection or uptake from the cell culture medium into the cells, or they are expressed in cells after transfection with plasmids or retroviruses or other vectors carrying an antisense gene. Antisense oligonucleotides were first discovered to inhibit viral replication or expression in cell culture for Rous sarcoma virus, vesicular stomatitis virus, herpes simplex virus type 1, simian virus and influenza virus. Since then, inhibition of mRNA translation by antisense oligonucleotides has been studied extensively in cell-free systems including rabbit reticulocyte lysates and wheat germ extracts. Inhibition of viral function by antisense oligonucleotides has been demonstrated *in vitro* using oligonucleotides which were complementary to the AIDS HIV retrovirus RNA (Goodchild, J. 1988 "Inhibition of Human Immunodeficiency Virus Replication by Antisense Oligodeoxynucleotides", *Proc. Natl. Acad. Sci. (USA)* **85**(15), 5507-11). The Goodchild study showed that oligonucleotides that were most effective were complementary to the poly(A) signal; also effective were those targeted at the 5′ end of the RNA, particularly the cap and 5′ untranslated region, next to the primer binding site and at the primer binding site. The cap, 5′ untranslated region, and poly(A) signal lie within the sequence repeated at the ends of retrovirus RNA (R region) and the oligonucleotides complementary to these may bind twice to the RNA.

Oligonucleotides are subject to being degraded or inactivated by cellular endogenous nucleases. To counter this problem, it is possible to use modified oligonucleotides, eg having altered internucleotide linkages, in which the naturally occurring phosphodiester linkages have been replaced with another linkage. For example, Agrawal *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7079-7083 showed increased inhibition in tissue culture of HIV-1 using

oligonucleotide phosphoramidates and phosphorothioates. Sarin *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7448-7451 demonstrated increased inhibition of HIV-1 using oligonucleotide methylphosphonates. Agrawal *et al* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7790-7794 showed inhibition of HIV-1 replication in both early-infected and chronically infected cell cultures, using nucleotide sequence-specific oligonucleotide phosphorothioates. Leither *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3430-3434 report inhibition in tissue culture of influenza virus replication by oligonucleotide phosphorothioates.

Oligonucleotides having artificial linkages have been shown to be resistant to degradation *in vivo*. For example, Shaw *et al* (1991) in *Nucleic Acids Res.* **19**, 747-750, report that otherwise unmodified oligonucleotides become more resistant to nucleases *in vivo* when they are blocked at the 3□ end by certain capping structures and that uncapped oligonucleotide phosphorothioates are not degraded *in vivo*.

A detailed description of the H-phosphonate approach to synthesising oligonucleoside phosphorothioates is provided in Agrawal and Tang (1990) *Tetrahedron Letters* **31**, 7541-7544, the teachings of which are hereby incorporated herein by reference. Syntheses of oligonucleoside methylphosphonates, phosphorodithioates, phosphoramidates, phosphate esters, bridged phosphoramidates and bridge phosphorothioates are known in the art. See, for example, Agrawal and Goodchild (1987) *Tetrahedron Letters* **28**, 3539; Nielsen *et al* (1988) *Tetrahedron Letters* **29**, 2911; Jager *et al* (1988) *Biochemistry* **27**, 7237; Uznanski *et al* (1987) *Tetrahedron Letters* **28**, 3401; Bannwarth (1988) *Helv. Chim. Acta.* **71**, 1517; Crosstick and Vyle (1989) *Tetrahedron Letters* **30**, 4693; Agrawal *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1401-1405, the teachings of which are incorporated herein by reference. Other methods for synthesis or production also are possible. In a

preferred embodiment the oligonucleotide is a deoxyribonucleic acid (DNA), although ribonucleic acid (RNA) sequences may also be synthesised and applied.

The oligonucleotides useful in the invention preferably are designed to resist degradation by endogenous nucleolytic enzymes. *In vivo* degradation of oligonucleotides produces oligonucleotide breakdown products of reduced length. Such breakdown products are more likely to engage in non-specific hybridization and are less likely to be effective, relative to their full-length counterparts. Thus, it is desirable to use oligonucleotides that are resistant to degradation in the body and which are able to reach the targeted cells. The present oligonucleotides can be rendered more resistant to degradation *in vivo* by substituting one or more internal artificial internucleotide linkages for the native phosphodiester linkages, for example, by replacing phosphate with sulphur in the linkage. Examples of linkages that may be used include phosphorothioates, methylphosphonates, sulphone, sulphate, ketyl, phosphorodithioates, various phosphoramidates, phosphate esters, bridged phosphorothioates and bridged phosphoramidates. Such examples are illustrative, rather than limiting, since other internucleotide linkages are known in the art. See, for example, Cohen, (1990) *Trends in Biotechnology*. The synthesis of oligonucleotides having one or more of these linkages substituted for the phosphodiester internucleotide linkages is well known in the art, including synthetic pathways for producing oligonucleotides having mixed internucleotide linkages.

Oligonucleotides can be made resistant to extension by endogenous enzymes by "capping" or incorporating similar groups on the 5' or 3' terminal nucleotides. A reagent for capping is commercially available as Amino-Link II™ from Applied BioSystems Inc, Foster City, CA. Methods for capping are

described, for example, by Shaw *et al* (1991) *Nucleic Acids Res.* **19**, 747-750 and Agrawal *et al* (1991) *Proc. Natl. Acad. Sci. USA* **88(17)**, 7595-7599, the teachings of which are hereby incorporated herein by reference.

A further method of making oligonucleotides resistant to nuclease attack is for them to be "self-stabilised" as described by Tang *et al* (1993) *Nucl. Acids Res.* **21**, 2729-2735 incorporated herein by reference. Self-stabilised oligonucleotides have hairpin loop structures at their 3' ends, and show increased resistance to degradation by snake venom phosphodiesterase, DNA polymerase I and fetal bovine serum. The self-stabilised region of the oligonucleotide does not interfere in hybridization with complementary nucleic acids, and pharmacokinetic and stability studies in mice have shown increased *in vivo* persistence of self-stabilised oligonucleotides with respect to their linear counterparts.

It will be appreciated that antisense agents also include larger molecules which bind to said interacting polypeptide mRNA or genes and substantially prevent expression of said interacting polypeptide mRNA or genes and substantially prevent expression of said interacting polypeptide. Thus, expression of an antisense molecule which is substantially complementary to said interacting polypeptide is envisaged as part of the invention.

The said larger molecules may be expressed from any suitable genetic construct as is described below and delivered to the patient. Typically, the genetic construct which expresses the antisense molecule comprises at least a portion of the said interacting polypeptide coding sequence operatively linked to a promoter which can express the antisense molecule in the cell. Suitable promoters will be known to those skilled in the art, and may include promoters for ubiquitously expressed, for example housekeeping genes or for

tissue-specific genes, depending upon where it is desired to express the antisense molecule.

Although the genetic construct can be DNA or RNA it is preferred if it is DNA.

Preferably, the genetic construct is adapted for delivery to a human cell.

Means and methods of introducing a genetic construct into a cell in an animal body are known in the art. For example, the constructs of the invention may be introduced into the cells by any convenient method, for example methods involving retroviruses, so that the construct is inserted into the genome of the (dividing) cell.

Other methods involve simple delivery of the construct into the cell for expression therein either for a limited time or, following integration into the genome, for a longer time. An example of the latter approach includes liposomes (Nassander *et al* (1992) *Cancer Res.* **52**, 646-653). Other methods of delivery include adenoviruses carrying external DNA via an antibody-polylysine bridge (see Curiel *Prog. Med. Virol.* **40**, 1-18) and transferrin-polycation conjugates as carriers (Wagner *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3410-3414). The DNA may also be delivered by adenovirus wherein it is present within the adenovirus particle. It will be appreciated that "naked DNA" and DNA complexed with cationic and neutral lipids may also be useful in introducing the DNA of the invention into cells of the patient to be treated. Non-viral approaches to gene therapy are described in Ledley (1995) *Human Gene Therapy* **6**, 1129-1144. Alternative targeted delivery systems are also known such as the modified adenovirus system described in WO 94/10323 wherein, typically, the DNA

is carried within the adenovirus, or adenovirus-like, particle. Michael *et al* (1995) *Gene Therapy* **2**, 660-668 describes modification of adenovirus to add a cell-selective moiety into a fibre protein. Mutant adenoviruses which replicate selectively in p53-deficient human tumour cells, such as those described in Bischoff *et al* (1996) *Science* **274**, 373-376 are also useful for delivering the genetic construct of the invention to a cell. Thus, it will be appreciated that a further aspect of the invention provides a virus or virus-like particle comprising a genetic construct of the invention. Other suitable viruses or virus-like particles include HSV, AAV, vaccinia and parvovirus.

A ribozyme capable of cleaving the interacting polypeptide RNA or DNA. A gene expressing said ribozyme may be administered in substantially the same and using substantially the same vehicles as for the antisense molecules. Ribozymes which may be encoded in the genomes of the viruses or virus-like particles herein disclosed are described in Cech and Herschlag "Site-specific cleavage of single stranded DNA" US 5,180,818; Altman *et al* "Cleavage of targeted RNA by RNAse P" US 5,168,053, Cantin *et al* "Ribozyme cleavage of HIV-1 RNA" US 5,149,796; Cech *et al* "RNA ribozyme restriction endoribonucleases and methods", US 5,116,742; Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endonucleases and methods", US 5,093,246; and Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endoribonucleases and methods; cleaves single-stranded RNA at specific site by transesterification", US 4,987,071, all incorporated herein by reference.

The genetic constructs of the invention can be prepared using methods well known in the art.

A further aspect of the invention provides a method of determining the susceptibility of a patient (preferably human) to cancer, particuarly skin cancer, still more particularly melanoma, comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer, particuarly skin cancer, still more particularly melanoma.

A further aspect of the invention provides a method of diagnosing cancer, particuarly skin cancer, still more particularly melanoma, in a patient (preferably human) comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer, particuarly skin cancer, still more particularly melanoma.

It will be appreciated that determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer may in itself be diagnostic of cancer or it may be used by the clinician as an aid in reaching a diagnosis.

A further aspect of the invention provides a method of predicting the relative prospects of a particular outcome of a cancer, particuarly skin cancer, still more particularly melanoma, in a patient (preferably human) comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer.

Thus, the method of the third aspect of the invention may be useful in prognosis or aiding prognosis. The method may be used as an adjunct to known prognostic methods such as histopathological examination of biopsy tissue or imaging.

It will be appreciated that determination of the level of the said PEPP in the sample will be useful to the clinician in determining how to manage the cancer in the patient.

The level of said PEPP which is indicative of cancer may be defined as the increased level present in known cancerous cells, for example melanoma cells, over known non-cancerous cells, for example normal skin cells. The level of said PEPP protein may be, for example, at least 1½ fold higher in cancerous cells, or it may be at least 2-fold or 3-fold higher.

In one preferred embodiment of the invention it is determined whether the level of said PEPP nucleic acid, in particular mRNA, is a level associated with cancer. Preferably, the sample contains nucleic acid, such as mRNA, and the level of said PEPP is measured by contacting said nucleic acid with a nucleic acid which hybridises selectively to said PEPP nucleic acid.

By "selectively hybridising" is meant that the nucleic acid has sufficient nucleotide sequence similarity with the said human nucleic acid that it can hybridise under moderately or highly stringent conditions, as discussed above. As is well known in the art, the stringency of nucleic acid hybridization depends on factors such as length of nucleic acid over which hybridisation occurs, degree of identity of the hybridizing sequences and on factors such as temperature, ionic strength and CG or AT content of the

sequence. Thus, any nucleic acid which is capable of selectively hybridising as said is useful in the practice of the invention.

Nucleic acids which can selectively hybridise to the said human nucleic acid include nucleic acids which have >95% sequence identity, preferably those with >98%, more preferably those with >99% sequence identity, over at least a portion of the nucleic acid with the said human nucleic acid. As is well known, human genes usually contain introns such that, for example, a mRNA or cDNA derived from a gene would not match perfectly along its entire length with the said human genomic DNA but would nevertheless be a nucleic acid capable of selectively hybridising to the said human DNA. Thus, the invention specifically includes nucleic acids which selectively hybridise to said PEPP mRNA or cDNA but may not hybridise to a said PEPP gene. For example, nucleic acids which span the intron-exon boundaries of the said PEPP gene may not be able to selectively hybridise to the said PEPP mRNA or cDNA.

Conveniently, the nucleic acid capable of selectively hybridising to the said human nucleic acid such as mRNA and which is used in the methods of the invention further comprises a detectable label.

By "detectable label" is included any convenient radioactive label such as ^{32}P, ^{33}P or ^{35}S which can readily be incorporated into a nucleic acid molecule using well known methods; any convenient fluorescent or chemiluminescent label which can readily be incorporated into a nucleic acid is also included. In addition the term "detectable label" also includes a moiety which can be detected by virtue of binding to another moiety (such as biotin which can be detected by binding to streptavidin); and a moiety, such as an enzyme, which can be detected by virtue of its ability to convert

a colourless compound into a coloured compound, or *vice versa* (for example, alkaline phosphatase can convert colourless o-nitrophenylphosphate into coloured *o*-nitrophenol). Conveniently, the nucleic acid probe may occupy a certain position in a fixed array and whether the nucleic acid hybridises to the said PEPP nucleic acid can be determined by reference to the position of hybridisation in the fixed array.

Primers which are suitable for use in a polymerase chain reaction (PCR; Saiki *et al* (1988) *Science* **239**, 487-491) are preferred. Properties of suitable PCR primers are discussed above.

The level of said PEPP protein may be determined in a sample in any suitable way. It is particularly preferred if the molecule which selectively binds to PEPP is an antibody, as discussed above.

The level of said PEPP which is indicative of cancer may be defined as the increased level present in known cancerous cells over known non-cancerous. The level may be, for example, at least 1½ fold higher in cancerous or metastatic cells, or it may be at least 2-fold or 3-fold higher.

By "the relative amount of said PEPP protein" is meant the amount of said VGSC protein per unit mass of sample tissue or per unit number of sample cells compared to the amount of said PEPP protein per unit mass of known normal tissue or per unit number of normal cells. The relative amount may be determined using any suitable protein quantitation method. In particular, it is preferred if antibodies are used and that the amount of said PEPP protein is determined using methods which include quantitative western blotting, enzyme-linked immunosorbent assays (ELISA) or quantitative immunohistochemistry.

Where *in vivo* imaging is used to detect enhanced levels of PEPP protein for diagnosis in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. See, for review, Morrison, Science 229:1202 (1985); Oi et al, BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al, EP 171496; Morrison et al, EP 173494; Neuberger et al, WO 8601533; Robinson et al, WO 870267 1; Boulianne et al., Nature 312:643 (1984); Neuberger et al, Nature 314:268 (1985).

Typical techniques for binding the above-described labels to antibodies are provided by Kennedy et al., Clin. Chim. Acta 70:1-31 (1976), and Schurs et al, Clin. Chim. Acta 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleintide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

A further aspect of the invention comprises a kit of parts useful for diagnosing cancer, especially melanoma, comprising an agent which is capable of use in determining the level of PEPP protein or nucleic acid in a sample. The agent may be a nucleic acid which selectively hybridises to PEPP nucleic acid or the agent may be a molecule which selectively binds to PEPP protein or the agent may be an agent useful in selectively assaying the activity of PEPP.

Preferably, the kit further comprises a control sample containing PEPP nucleic acid or protein wherein the control sample may be a negative

control (which contains a level of PEPP protein or nucleic acid which is not associated with cancer) or it may be a positive control (which contains a level of PEPP protein or nucleic acid which is associated with cancer). The kit may contain both negative and positive controls. The kit may usefully contain controls of PEPP protein or nucleic acid which correspond to different amounts such that a calibration curve may be made.

The invention will now be described in detail with reference to the following Examples and Figures:

Figure Legends

Figure 1. SDS Polyacrylamide gel of purified GST-PH domains. 2 μg of the indicated purified GST PH domain fusions, except for TAPP1[W281L] mutant (0.5 μg), which expressed poorly, were electrophoresed on a 4-12% SDS polyacrylamide gel and stained with Coomassie blue. The positions of the molecular mass markers (Biorad Precision markers) are indicated. TAPP1, TAPP2, centaurin-β2 and pleckstrin-2 constructs were expressed in 293 cells and FAPP1, PEPP1, AtPH1, LL5α, LL5β, evectin-2 and PH30 were expressed in *E.coli*.

Figure 2. Phosphoinositide binding properties of the novel PH domains. The ability of the indicated GST fusion proteins to bind a variety of phosphoinositides was analysed using a protein-lipid overlay. Serial dilutions of the indicated phosphoinositides (100 pmol, 50 pmol, 25 pmol, 12.5 pmol, 6.3 pmol, 3.1 pmol and 1.6 pmol) were spotted onto a nitrocellulose membranes which were then incubated with the purified GST fusion proteins. The membranes were washed and the GST-fusion proteins

bound to the membrane by virtue of their interaction with lipid were detected using a GST antibody. A representative of at least 3 separate experiments carried out is shown.

Figure 3. Amino acid sequence and tissue distribution of TAPP1 and TAPP2. (A) The alignment of the human and mouse TAPP1 and TAPP2 sequences are shown. The identities are shaded in black. The DNA sequences encoding the human (h) and mouse (m) TAPP1 shown are available from the NCBI database (accession numbers for human TAPP1 AF286160, mouse TAPP1 AF286165, human TAPP2 AF286164 and mouse TAPP2 AF286161). The amino acid residues corresponding to the N-terminal and C-terminal PH domains are indicated by a solid line and a dotted line respectively. The residues that comprise the putative SH3 domain binding proline rich motif of TAPP2 are boxed. The residues of the C-terminal PH domain of TAPP1 and TAPP2 that make up the PPBM are marked indicated (+). The C-terminal Ser-Asp-Val sequence of TAPP1 and TAPP2 that could interact with proteins possessing a PDZ domain(s) is marked with asterisks. The sequence of mouse TAPP1 and human TAPP2 is a partial sequence and the residues that are not known are indicated by a blank space. (B); TAPP1 and TAPP2 cDNAs were labelled with ^{32}P using random primers (see experimental section) and used to probe a Northern blot containing polyA+ RNA isolated from the indicated human tissues and cancer cell lines. The blot was washed and autoradiographed. The TAPP1 and TAPP2 probes were observed to hybridise to a 4 kb and a 6 kb message, respectively.

Figure 4. Comparison of the phosphoinositide binding properties of the N-terminal and C-terminal PH domains of TAPP1 and TAPP2. The ability of wild type and mutant forms of full length (FL) and isolated N-terminal (NT) and C-terminal (CT) PH domains of TAPP1 and TAPP2 GST-fusion proteins to interact with phosphoinositides were analysed using a protein-lipid overlay. Serial dilutions of the indicated phosphoinositides (100 pmol, 50 pmol, 25 pmol, 12.5 pmol, 6.3 pmol, 3.1 pmol and 1.6 pmol) were spotted onto a nitrocellulose membrane which was then incubated with the indicated purified GST fusion proteins. The membranes were washed and the GST-fusion proteins bound to the membrane by virtue of their interactions with lipid were detected using a GST antibody. A representative experiment of three is shown. The isolated N-terminal PH domain of human TAPP1 comprises residues 1 to 147, the isolated C-terminal PH domain of human TAPP1 comprises residues 95 to 404, the isolated N-terminal PH domain of mouse TAPP2 comprises residues 1 to 131 and the isolated C-terminal PH of mouse TAPP2 comprises residues 174 to 425.

Figure 5 Amino acid sequence of human and mouse FAPP1. The alignment of the full length human and mouse FAPP1 and partial Xenoupus and zebrafish sequences are shown. The identities are shaded in black. The DNA sequences of human (accession number AF286162) and mouse FAPP1 (accession number AF286163) are available from the NCBI database. The partial *Xenopus* and *zebrafish* FAPP1 sequences are predicted from the EST sequences with NCBI accession numbers AW644282 and AW174299 respectively. The amino acid residues corresponding to the PH domain are underlined and the residues that comprise the putative SH3

domain binding motif are indicated by a dotted line. The residues of the PH domain of FAPP1 that make up the PPBM are marked indicated (+).

Figure 6. Amino acid sequence and tissue distribution of PEPP1. (A) The partial sequence of human PEPP1 that has been sequenced thus far is shown. The amino acid residues corresponding to the PH domain are indicated by a solid line and the residues that could form a putative SH3 domain binding motif are indicated by a dotted line. The DNA sequence is available from the NCBI database (accession number AF286166). The residues of the PH domain of PEPP1 that make up the PPBM are marked indicated (+). (B) The partial cDNA for PEPP1 shown above was labelled with ^{32}P, using random primers, and used to probe a Northern blot containing polyA+ RNA isolated from the indicated human tissues and cancer cell lines. The blot was washed and autoradiographed. The PEPP1 probe was observed to hybridise with a 3 kb message in the melanoma G-361 cell line.

Figure 7. Alignment of PH domains. Identities are indicated in black and homolgies in grey. Residues making up the PPBM are indicated with asterisks. Abbreviations: h, human; m, mouse; b2-cent, β2-centaurin.

Figure 8: Amino acid sequence and tissue distribution of PEPP1, 2 and 3. (A) The alignment of the full length human sequences of PEPP1, PEPP2 and PEPP3 are shown. The identities are shaded in black. The DNA sequences of human PEPP1 and human PEPP3 are indicated above and in NCBI database entries AF286166 and NM_014935. The amino acid residues corresponding to the PH domain are indicated by a solid line and

the region of homology preceding the PH domain is indicated with a dotted line. The residues of the PH domain of PEPP1 that make up the PPBM are marked indicated (+) and the WW domains of PEPP2 are boxed. (B) The partial cDNA for PEPP1 and PEPP2 shown above was labelled with ^{32}P using random primers and used to probe a Northern blot containing polyA$^+$ RNA isolated from the indicated human tissues and cancer cell lines. The blot was washed and autoradiographed. The PEPP1 probe was observed to hybridise with a 3kb message in the melanoma G-361 cell line and the PEPP2 probe hybridised with a 4.6 kb message.

Figure 9: Amino acid and nucleotide sequences of human FAPP2.

Figure 10: Amino acid sequecne alignment of human FAPP1 and human FAPP2.

Figure 11: Human FAPP2 specifically binds phosphoinositol 4-monophosphate (PtdIns-4P). Methods used are equivalent to those specified in the legend to Figure 2.

Example 1: Identification of PH domains with novel phosphoinositide binding specificities.

The second messsenger phospatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P_3) is generated by the action of phosphatidylinositol 3-kinase (PI 3-kinase) and regulates a plethora of cellular processes. An approach for dissecting the mechanisms by which these processes are regulated, is to identify proteins that interact specifically with PtdIns(3,4,5)P_3. The pleckstrin homology (PH) domain has become recognised as the specialised

module used by many proteins to interact with PtdIns(3,4,5)P_3. Recent work has led to the identification of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM) at the N-terminal regions of PH domains that interact with this lipid. We have identified novel or uncharacterised PH domains possessing a PPBM and determined their phosphoinositide binding properties. Surprisingly, many of the PH domains identified possess unexpected phosphoinositide binding specificities and do not bind PtdIns(3,4,5)P_3. These include PH domains that interact specifically with PtdIns(3,4)P_2 (TAPP1), PtdIns3P (PEPP1 & AtPH1 and also PEPP2 and PEPP3), PtdIns4P (FAPP1) and PtdIns(3,5)P_2 (Centaurin-β2).

Abbreviations: ARF, ADP ribosylation factor; DAPP1, dual adaptor for phosphotyrosine and 3-phosphoinositides; EST, expressed sequence tag; FAPP1, PtdIns-Four-phosphate AdaPtor Protein-1; GAP, GTPase activating protein; GST, glutathione-S-transferase; NCBI, National Center for Biotechnology Information; PKC, protein kinase C; PDZ, postsynaptic density protein (PSD-95)/Drosophila disc large tumour suppressor (Dlg)/tight junction protein (ZO1); PDK1, 3-phosphoinositide-dependent protein kinase-1; PH, pleckstrin homology; PEPP1, PtdIns-thrEe-Phosphate binding PH domain Protein-1; PI 3-kinase, phosphoinositide 3-kinase; PKB, protein kinase B; PPBM, Putative PtdIns(3,4,5)P_3 binding motif; PtdIns, phosphatidylinositol; TAPP, TAndem PH domain containing Protein; Xaa, any amino acid.

Materials All phosphoinositides used in this study were dipalmitoyl derivatives obtained from Cell Signals, which were analysed by thin layer chromatography and found to migrate as single products. Hybond-C extra was from Amersham Pharmacia Biotech, High Fidelity PCR kit from Roche, Human tissue (Catalogue number 7780-1), mouse tissue (Catalogue

number 7762-1) and human cancer cell line (Catalogue number 7757-1) Multiple Tissue Northern Blots from Clontech, Human Universal cDNA Library was from Strategene, pCR 2.1Topo vector and precast SDS polyacrylamide gels were from InVitrogen. DAPP1 and Grp1 [8] were expressed as fusion proteins with glutathione-S-transferase (GST) in 293 cells [4]. The PH domain of human phospholipase Cδ1 (residues 20 to 184) fused to GST was expressed in *E. coli.*

General methods and buffers. Restriction enzyme digests, DNA ligations, site directed mutagenesis and other recombinant DNA procedures were performed using standard protocols, as well known to those skilled in the art. All DNA constructs were verified by DNA sequencing.
Buffer A : 50 mM Tris-HCl pH 7.5, 1 mM EGTA, 1 mM EDTA, 1% (by mass) Triton-X 100, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 5 mM sodium pyrophosphate, 0.27 M sucrose, 1 μM microcystin-LR, 0.1% (by vol) β-mercaptoethanol and 'complete' proteinase inhibitor cocktail (one tablet per 50 ml, Roche). Buffer B: 50 mM Tris/HCl pH 7.5, 0.1 mM EGTA, 10 mM β-mercaptoethanol and 0.27M sucrose.

Cloning of PH domains and preparation of expression constructs. All the human and mouse EST's were obtained from the I.M.A.G.E. Consortium [13] and sequenced. The plant EST (accession number T04439) encoding a full length clone of AtPH1 was obtained from the Arabidopsis Biological Research Centre (Ohio University). The sequence of each EST was verified and the full length PH domain of each EST was amplified by PCR using the Hi-fidelity PCR system with primers designed to incorporate a Kozak site, an initiating ATG codon

followed by a myc epitope tag and a stop codon after the PH domain. The region of each protein that was amplified using the indicated EST as template was as follows: human TAPP1 (residues 95 to 404, accession number AI216176), mouse TAPP2 (residues 174 to 425, accession number AA111410), human FAPP1 (residues 1 to 99, accession number W32183), *Arabidopsis thaliana* AtPH1 (full length protein, residues 1 to 145, accession number, T04439), human PEPP1 (sequence in Fig 6 Ser-Ala-Ser to Arg-Pro-Gln, accession number N31123), mouse centaurin-β2 (residues 266 to 390, accession number AA967911), putative human homologue of rat LL5α (sequence Ser-Glu-Ser-Ala to Gln-Phe-Met-Asn, accession number AA863428), putative human isoform of LL5α which we have termed LL5β (sequence Arg-Lys-Glu-Asp to His-Phe-Leu-Leu, accession number AA461369), mouse pleckstrin-2 (residues 1 to 249, accession number AI326844), human evectin-2 (residues 1 to 167, accession number AA101447) and human PH30 (sequence Asn-Ser-Ser-Ile to Ile-Ser-Asp-Ala, accession number AI827615). The PCR products were resolved on 1% agarose, gel purified, cloned into the pCR2.1 TOPO vector, sequenced and subcloned into the *E.coli* pGEX-4T-1 expression vector or the mammalian pEBG2T vector that codes for the expression of these proteins with a GST tag at the N-terminus.

Expression of GST-PH domains in *E.coli*. The pGEX-4T-1 constructs encoding the PH domains of FAPP1, AtPH1, PEPP1, LL5α, LL5β, evectin-2 and PH30 were transformed into BL21 *E.coli* cells and a 0.5L culture was grown at 37 °C in Luria Broth containing 100 μg/ml ampicillin, until the absorbance at 600 nm was 0.6. 250 μM isopropyl-β-D-galactosidase was

added and the cells cultured for a further 16 h at 26 °C. The cells were resuspended in 25 ml of ice-cold Buffer A and lysed by one round of freeze thawing and the lysates sonicated to fragment the DNA. The lysates were centrifuged at 4°C for 30 min at 20, 000 x g, the supernatant filtered through a 0.44 micron filter and incubated for 60 min on a rotating platform with 1 ml of glutathione-Sepharose previously equilibrated in Buffer A. The suspension was centrifuged for 1 min at 3000 x g, the beads washed three times with 15 ml of Buffer A containing 0.5 M NaCl, and then a further ten times with 15 ml of Buffer B. The protein was eluted from the resin at ambient temperature by incubation with 2 ml of Buffer B containing 20 mM glutathione, and the beads removed by filtration through a 0.44 micron filter. The eluate was divided into aliquots, snap frozen in liquid nitrogen, and stored at -80°C.

Expression of GST-PH domains in human embryonic kidney 293 cells. As the PH domains of TAPP1, TAPP2, centaurin-β2, and pleckstrin-2 were significantly degraded when expressed in bacteria (data not shown), these were expressed as GST fusion proteins in human embryonic kidney 293 cells. For the expression of each construct, twenty 10 cm diameter dishes of 293 cells were cultured and each dish transfected with 5 μg of the pEBG-2T construct, using a modified calcium phosphate method [14]. 36 h post-transfection, the cells were lysed in 1 ml of ice-cold Buffer A, the lysates pooled, centrifuged at 4°C for 10 min at 13, 000 x g and the GST-fusion proteins were purified by affinity chromatography on glutathione-Sepharose and stored as described above.

Cloning TAPP1, TAPP2 , FAPP1 and PEPP1. Full length human TAPP1, full length mouse TAPP2, partial mouse TAPP1, partial human TAPP2, and full length human and mouse FAPP1 sequences were deduced by sequencing the EST clones listed in Table 3. Several EST clones possessed identical sequences, and had the same in-frame stop codon 5' to the predicted initiating ATG codon and possessed a stop codon at the same position at the 3' end of the gene. The constructs used to express full length and deletion mutants of TAPP1 and TAPP2 were generated by PCR, using as a template ESTs encoding full length human TAPP1 (accession number AI216176) and full length mouse TAPP2 (accession number AA111410). The PCR primers used were designed to incorporate a Kozak site, and an initiating ATG codon followed by a Flag epitope tag and the resulting PCR product was subcloned into the pEBG2T mammalian expression vector.

Cloning of PEPP1 and FAPP1. A Stratagene Human Universal cDNA Library was screened with a DNA probe corresponding to the PH domains of PEPP1 and FAPP1 and we were able to isolate a clone encoding each of these proteins using this approach. The partial sequence of PEPP1 that contains the 5'end of the coding sequence was obtained by sequencing of ESTs with NCBI accession numbers N49341and N31123. To obtain a full length cDNA encoding PEPP1, we screened a Stratagene Human Universal cDNA Library with a DNA probe corresponding to the N-terminal 15 to 169 residues of PEPP1 and we isolated a full length PEPP1 cDNA which had a stop codon 5' to the predicted initiating ATG codon an open reading frame encoding 779 amino acids followed by a stop codon. Interrogation of the EST databases with the full length PEPP1 sequ4ence identified 2 closely related isoforms of this protein termed PEPP2 and PEPP3. The sequence of human PEPP2 was deduced by sequencing the following EST clones:

A1808805 (kidney), AA232124(brain), W91917 (foetal liver and spleen) and AI638629 (germ cell line). The sequence of PEPP2 is likely to be full length as there is a stop codon 5' to the predicted initiating ATG codon. ESTs relating to PEPP3 are AI739438, BE303674 and F23241.

Northern Blot Analysis. cDNA corresponding to full length human TAPP1, partial human TAPP2 (residues 18 to 304), partial human PEPP1 (residues encoding sequence Ser-Ala-Ser to Arg-Pro-Gln), partial human PEPP2 (residues 154 to 654) and mouse partial mouse centaurin-β2 (residues 266 to 390) were ^{32}P-labelled by random priming using a multi-prime DNA labelling kit (Amersham Pharmacia). These probes were then used to screen Northern blots using Rapid-Hyb Buffer (Amersham Pharmacia) according to the protocol provided by the manufacturer.

Protein-Lipid overlay. To assess the phosphoinositide binding properties of each PH domain, a protein-lipid overlay assay was performed using the GST fusion proteins as described previously [4, 15]. Briefly, 1 µl of lipid solution containing 1-100 pmol of phospholipids dissolved in a mixture of choroform:methanol:water (1:2:0.8) was spotted onto Hybond-C extra membrane and allowed to dry at room temperature for 1 h. The membrane was blocked in 3% (by mass) fatty acid-free BSA in TBST (50 mM Tris/HCl pH 7.5, 150 mM NaCl and 0.1% Tween-20 (by vol) for 1h. The membrane was then incubated overnight at 4 °C with gentle agitation in the same solution containing 0.2 µg/ml of the indicated GST fusion protein. The membranes were washed 6 times over 30 min in TBST and then incubated for 1h with 1/1000 dilution of anti-GST monoclonal antibody (Sigma). The membranes were washed as before, then incubated for 1h with

1/5000 dilution of anti-mouse-HRP conjugate (Pierce). Finally, the membranes were washed 12 times over 1h in TBST and the GST-fusion protein bound to the membrane by virtue of its interaction with phospholipid was detected by enhanced chemiluminescence.

BIACore measurements of PH domain-lipid interactions.

Kinetic analyses of the interactions between the GST PH domain fusions and the polyphosphoinositides were made using surface plasmon resonance based procedures as described previously [4, 16], with the following modifications. The mole percentage of the test polyphosphoinositide was reduced from 1 % to 0.1%. This helped to minimise any mass transport limitation in the binding interaction and increased the rate of lipid immobilisation on the chip. The intracellular buffer was supplemented to 0.27 M sucrose to reduce the bulk refractive index changes associated with the addition of Buffer B. Proteins were injected over the monolayers at concentrations ranging from 1 μM to 10 nM. Data were analysed using the bimolecular interaction model and the global fitting feature of the BIAevaluation 3 software for several sensorgrams at different protein concentrations. GST PH domain binding to phosphoinositides does not fit well to this model due to the slow dissociation of the protein from the surface [4, 16]. Therefore, the affinity of binding of these proteins to polyphosphoinositides is likely to be overestimated by this method and the results are therefore stated as apparent equilibrium dissociation constants for comparative purposes. The relative binding affinities of each protein relative to the binding of full length GST-TAPP1 to PtdIns(3,4)P_2 were also calculated.

Results.

Identification of novel or uncharacterised PH domains. The NCBI/EMBL/PDB EST databases were interrogated with the amino acid sequences encoding the PH domains of human PKBα, PDK1, Grp1 and DAPP1. These searches revealed 11 partial sequences (see Table 1) encoding either novel or previously uncharacterised PH domain-containing proteins possessing at least 5 of the 6 conserved residues in the PPBM (Table 1). We cloned the entire PH domain of each of these proteins (see experimental section) which are named in Table 1. They were expressed in *E. coli* or human embryonic 293 cells as fusions to glutathione S-transferase (GST) and purified by affinity chromatography on glutathione-Sepharose. Homogeneous Coomassie blue-staining bands were observed for each product and these proteins migrated with the expected molecular masses on SDS-polyacrylamide gel electrophoresis (Fig 1).

We studied the specificity and affinity of interaction of the PH domains for phosphoinositide lipids using either a "protein-lipid overlay" assay [4] (Fig 2) or the more quantitative surface plasmon resonance based approach [16] (Table 2). For the protein-lipid overlay assay, serial dilutions of phosphoinositides were spotted on to a nitrocellulose membrane and incubated with the indicated GST PH domain fusion protein or GST-DAPP1 (that binds PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 [4]), GST-GRP1 (that binds only PtdIns(3,4,5)P_3 [8]) and GST-phospholipase Cδ1 (that binds only PtdIns(4,5)P_2 (Ferguson *et al* (1995) *Cell* **83**, 1037-1046)) as controls. The membranes were then washed and immunoblotted with a GST antibody to detect GST fusion

proteins bound to the membrane by virtue of their interaction with lipid. For the surface plasmon resonance based assay, the apparent K_d values of the GST PH domain fusion proteins resulting from their interaction with a supported lipid monolayer containing a low mole fraction of phosphoinositide, was determined (Table 2). Both these assays yielded comparable results for the lipid binding specificities and relative affinities of the PH domains that we have isolated. As discussed below, 6 of the PH domains we identified, did not bind to PtdIns(3,4,5)P_3 or *sn*-1-stearoyl-2-arachidonyl-D-PtdIns(3,4,5)P_3 (data not shown), but interacted with other phosphoinositides with varying affinity and specificity. In contrast, the PH domains derived from proteins termed LL5α [17], a previously undescribed closely related isoform to LL5α which we have termed LL5β, pleckstrin-2 [18, 19], and a protein that we have called PH30, which displays 70% identity to the nuclear dual-specificity phosphatse [20] (accession number AAC39675), interacted with several phosphoinositides (Fig 2). The PH domain of a protein of unknown function, termed evectin-2, which localises to post-golgi membranes [21] showed moderate affinity for PtdIns(3,4,5)P3 but also interacted more weakly with several other phosphoinositides (Fig 2). None of the PH domains whose lipid binding properties were investigated in Fig 2, interacted with phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine or phospatidylinositol in the protein-lipid overlay assay (data not shown).

TAPP1 and TAPP2 bind specifically to PtdIns(3,4)P_2. Two of the novel sequences identified encoded related proteins which were termed TAPP1 and TAPP2 (Table 1). Clones encoding the full length human TAPP1

(accession number AF286160) and mouse TAPP2 (accession number AF286161) as well as a partial mouse TAPP1 (accession number AF286165) and human TAPP2 (accession number AF286164), were isolated as described in the Methods section. Human TAPP1 is a protein of 404 amino acids and mouse TAPP2 is a protein of 425 amino acids (Fig 3A). A stop codon immediately 5' to the predicted initiating ATG codon indicates that both human and mouse TAPP1 and TAPP2 protein sequences are full length. Analysis of the TAPP1 and TAPP2 sequences revealed the presence in each protein of two PH domains, of which only the C-terminal PH domain possesses the PPBM (Fig 3A). Hence these proteins were termed TAPP for TAndem PH domain containing Protein. The amino acid sequences of TAPP1 and TAPP2 are 58% identical over the first 300 amino acids, which encompasses both of the PH domains. There is little homology between the C-terminal 100 residues of TAPP1 and TAPP2, except that 7 out of the 11 C-terminal amino acids of TAPP1 and TAPP2 are identical. The last 3 residues of TAPP1 and TAPP2 conform to the minimal sequence motif (Ser/Thr-Xaa-Val/Ile [22, 23]) required for binding to a PDZ domain. Apart from two proline rich regions towards the C-terminus of TAPP2, which could form a binding site for an SH3 domain (Fig 3), no other known catalytic domains are present. Interrogation of the NCBI human genome database with the TAPP1 sequence indicated that it is located on chromosome 10q25.3-q26.2. Although the genomic fragment that encompases TAPP2 (accession number AC067817) has been sequenced, its chromosomal location is not yet known.

The isolated C-terminal PH domains of TAPP1 and TAPP2 (which possess the PPBM), when expressed as GST-fusion proteins, interacted with PtdIns(3,4)P_2 but did not bind to PtdIns(3,4,5)P_3 or any other

phosphoinositides tested (Fig 2). Surface plasmon resonance studies indicated that the isolated C-terminal PH domain of TAPP1 and TAPP2 interacted with PtdIns(3,4)P_2 with apparent Kd values of 5 nM and 30 nM, respectively (Table 2). The N-terminal PH domain of TAPP1 and TAPP2 failed to interact with any phosphoinositide tested (Fig 4A and Table 2). The full length GST-TAPP1 (Fig 4A and Table 2) and full length GST-TAPP2 (Fig 4 C and Table 2) interacted specifically with PtdIns(3,4)P_2. Mutation of the conserved Arg212 to Leu in the PPBM of the C-terminal PH domain of TAPP1 abolished the interaction of both full length TAPP1 and the isolated C-terminal PH domain with PtdIns(3,4)P_2 (Fig 4B). Mutation to Leu of the residue (Arg28) in the N-terminal PH domain of TAPP1 that lies in the equivalent position to Arg212 in the C-terminal PH domain, did not affect the interaction of full length GST-TAPP1 with PtdIns(3,4)P_2 (Fig 4B). As expected, the mutation to Leu of the conserved Trp residue (Trp281) found in all PH domains, abolished the interaction of the isolated C-terminal PH domain of TAPP1 with PtdIns(3,4)P_2 (Fig 4B).

The tissue distribution of TAPP1 and TAPP2 mRNA was investigated by Northern blot analysis. TAPP1 was detected as a 4 kb transcript in all tissues examined with the highest levels observed in skeletal muscle, spleen, lung, thymus and placenta (Fig 3B). TAPP2 was detected as a 6 kb transcript in all tissues examined with the highest levels observed in heart and kidney (Fig 3B). We identified many ESTs encoding TAPP1 and TAPP2 in the databases derived from several tissues (Table 3), indicating that TAPP1 and TAPP2 are widely expressed proteins.

FAPP1 is a specific PtdIns4P binding protein. The identified PH domain termed FAPP1 (Table 1), possessing Gln instead of Lys or Arg at the third conserved residue of the PPBM, exhibited a high affinity for PtdIns4P (K_d 20 nM), but did not bind to any other phosphoinositide (Fig 2 & Table2). The full length human and mouse FAPP1 sequences (Fig 5) were deduced from the sequencing of ESTs listed in Table 3. Human FAPP1 encodes a protein of 300 amino acids and a stop codon immediately 5' to the predicted initiating ATG codon indicates that both the human and mouse FAPP1 protein sequences are full length. Interrogation of the human genome NCBI database indicated that the FAPP1 gene was located on an unmapped region of chromosome 2 (accession number NT_003398). Analysis of the FAPP1 sequence revealed the presence of an N-terminal PH domain and a proline rich region located towards the C-terminus that could mediate binding to SH3 domains (Fig 5). FAPP1 is likely to be expressed widely, because 27 EST clones encoding this protein were derived from several tissues (Table 3). However, FAPP1 may not be an abundant transcript as we were unable to detect significant levels of FAPP1 mRNA expression in any tissue or cell line examined (data not shown).

FAPP2 also binds specifically to PtdIns4P.

Plant AtPH1 and mammalian PEPP bind PtdIns3P specifically. Two of the PH domains that were identified, termed AtPH1 and PEPP1 (Table 1), exhibited significant affinity for PtdIns3P (K_d of 325 nM), but did not bind to any other phosphoinositide (Fig 2 and Table 2). AtPH1 is a small 145 residue *Arabidopsis* protein, whose physiological role is unknown. It consists of one PH domain with a short N-terminal extension and is

expressed in all plant tissues [24]. PEPP1 is a novel mammalian protein, whose partial sequence (Fig 6A) and full length sequence (Fig 8A) we have deduced from sequencing of several ESTs (Table 3). The partial sequence is likely to comprise the N-terminal end of PEPP1 as there is an in-frame stop codon 5' to the predicted initiating ATG codon. The PH domain of PEPP1 is located at the N-terminal region of PEPP1. There are also 2 proline rich regions that could comprise SH3 binding sites. Analysis of the NCBI human genome database shows that the PEPP1 gene is located on an unmapped region of chromosome 19 (accession number AC026803). The tissue distribution of PEPP1 mRNA was first investigated by Northern blot analysis, which indicated that PEPP1 was either not expressed or only expressed to a very low level in the panel of 12 tissues that we examined (Fig 6B). We also carried out a Northern blot analysis using a panel of 8 different human cancer cell lines (Fig 6B). Interestingly, PEPP1 mRNA was expressed at very high levels in a melanoma cancer cell line as a 3 kb fragment, but was not significantly expressed in the other 7 non-melanoma cancer cell lines that were investigated (Fig 6B). Further evidence which suggests that PEPP1 may be selectively expressed in melanoma or melanocytes is that the three human EST clones encoding PEPP1 that we have identified thus far are derived from either a melanoma or a melanocyte cDNA library (Table 3).

Interrogation of the NCBI database with the PEPP1 sequence revealed 2 other proteins that appear to be related isoforms of PEPP1 termed PEPP2 and PEPP3. The identity between these proteins is most notable in the PH domain, especially in the region that encompasses the PPBM as well as a region of 30 amino acids that precedes the PH domain. PEPP1, PEPP2 and PEPP3 are poorly conserved in the region C-terminal to the PH domain (Fig

8A). PEPP2, but not PEPP1 or PEPP3 also possesses two WW domains (Rotin (1998) *Curr Top Microbiol Immunol* **228**, 115-133) in a region N-terminal to the PH domain (Fig 8A). PEPP2 may be more widely expressed than PEPP1 as Northern Blot analysis shows that PEPP2 mRNA is present in high levels in heart and kidney and also expressed at a lower level in other tissues. PEPP3 may not be an abundant transcript as we were unable to detect significant levles of PEPP3 mRNA expression in any tissue or cell line examined (data not shown). The four PEPP3 ESTs that are present in the database are derived from brain, colon, mammary gland and skeletal muscle (see methods). PEPP2 and PEPP3 are also considered to bind PtdIns3P.

Centaurin-β2 is a PtdIns(3,5)P_2 binding protein. Human centaurin-β2 is an uncharacterised 778 amino acid protein (cloned by T. Jackson and colleagues, University College London, accession number CAB41450), possessing a PH domain (residues 267-363) followed by a putative ARF GAP domain (residues 399-520) and three ankyrin repeats at its C-terminus. The PH domains of both mouse and human centaurin-β2 possess Asn instead of a Lys or Arg at the third conserved residue of the PPBM (Table 1). The PH domain of mouse centaurin-β2 exhibited moderate affinity for PtdIns(3,5)P_2 but did not bind to any other phosphoinositide tested (Fig 2). Centaurin-β2 is likely to be a widely expressed protein as 12 EST clones encoding it were derived from several tissues and Northern blot analysis indicated that mouse centaurin-β2 was expressed as a 4.5 kb fragment in all tissues investigated (data not shown).

Discussion

The PH domains identified thus far that bind specifically to PtdIns(3,4,5)P_3, or to PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2, possess a PPBM (Table 1). However, the finding in this study that PH domains possessing a perfect or near perfect PPBM consensus, do not always interact with PtdIns(3,4,5)P_3 specifically, emphasises that residues lying outside the PPBM also influence the interaction of many PH domains with phosphoinositides. It therefore seems unlikely that it will be possible to predict the lipid binding specificity of a PH domain based on its primary amino acid sequence alone. This is consistent with structural studies showing that residues lying outside of the PPBM also form direct contacts with the inositol phosphate moieties of phosphoinositides [12, 25]. Previous studies have demonstrated that PLCδ_1 which also possesses a PPBM, does not bind to PtdIns(3,4,5)P_3 with high affinity [25]. It has been proposed that, in this case, the short loop between the β1 and β 2 strands of the PH domain of PLCδ_1 compared to that found in other PH domains that bind to PtdIns(3,4,5)P_3, may account for this observation [25].

There has been considerable debate as to whether PtdIns(3,4)P_2 regulates the same physiological processes as PtdIns(3,4,5)P_3, as it is formed as a breakdown product of PtdIns(3,4,5)P_3 and many of the PH domains that interact with PtdIns(3,4,5)P_3 also bind to PtdIns(3,4)P_2 (as discussed in the introductory section above). However, the finding that agonists such as hydrogen peroxide, [26] and crosslinking of platelet integrin receptors [27], elevate PtdIns(3,4)P_2 without increasing PtdIns(3,4,5)P_3, suggest that PtdIns(3,4)P_2 may be able to regulate physiological processes distinct from those controlled by PtdIns(3,4,5)P_3. TAPP1 and TAPP2 (Fig 3) are the first

proteins to be identified that interact with PtdIns(3,4)P_2 specifically and may therefore be key mediators of cellular responses that are regulated specifically by this second messenger. Although, there are no apparent homologues of TAPP1 and TAPP2 present in the completed genome of *Drosophila*, *C.elegans* or *S. cerevisiae*, there are ESTs encoding a TAPP1 homologue derived from zebrafish and chicken (Table 3). Further studies are required to characterise the physiological role of TAPP1 and TAPP2, but it is possible that they function as adaptor proteins to recruit proteins that interact with them to cellular membranes in response to extracellular signals that lead to the generation of PtdIns(3,4)P_2. However, it is possible that the *in vitro* lipid binding properties of TAPP1 and TAPP2, as well as the other PH domain containing proteins that we have characterised in this study, could differ from their *in vivo* binding specificities. It is also possible that the inositol polyphosphate head groups of the phosphoinositides, rather than the phosphoinositides themselves, could be the natural ligands for these proteins. The N-terminal PH domain of TAPP1 and TAPP2, rather than interacting with lipids, may mediate protein-protein interactions as they did not interact with any phosphoinositide that we tested (Fig 4A). TAPP1 and TAPP2 could also potentially interact with proteins containing PDZ domains through their C-terminal Ser-Xaa-Val residues and TAPP2 could bind to SH3 domains through two proline rich motifs located towards its C-terminus.

To our knowledge, the only PH domain previously shown to bind PtdIns4P with some specificity is derived from a plant PtdIns 4-kinase which also interacts weakly with PtdIns(4,5)P_2 [28]. In contrast, FAPP1 (Fig 5) only binds PtdIns 4P and does not interact with PtdIns(4,5)P_2 (Fig 2, Table 2). A

key role of PtdIns 4P in mammalian cells is to act as an intermediate in the synthesis of PtdIns(4,5)P_2. Apart from a PH domain and a putative SH3-binding proline-rich motif, FAPP1 does not possess a catalytic domain that would indicate a role in regulating the synthesis or breakdown of PtdIns4P in cells. There are no apparent homologues of FAPP1 in *Drosophila*, *C.elegans* or *S. cerevisiae*; however ESTs encoding FAPP1 have been identified in zebrafish and *Xenopus* (Fig 5 and Table 3).

Genetic studies carried out in yeast have demonstrated that PtdIns3P plays an important role in regulating golgi to vacuole or lysosome membrane trafficking as well as endosome function [29]. Several proteins (e.g. EEA1) regulating these processes have been found to interact with PtdIns3P through a particular type of Zinc finger domain (known as the FYVE domain) [30]. To our knowledge the only other PH domain-containing protein other than PEPP1 and AtPH1, previously reported to interact with PtdIns3P is phospholipase Cβ1[31]. However phospholipase Cβ1 may be less specific for PtdIns3P than PEPP1 and AtPH1, as it also possessed significant affinity for PtdIns(4,5)P_2 and PtdIns(3,4,5)P_3 [31]. The evidence indicates that phospholipase Cβ1 may be recruited to plasma membranes through an interaction of its PH domain with both PtdIns 3P (or other phosphoinositide) and the Gβγ regulatory subunits [31, 32].

A potentially interesting feature of PEPP1, is that its expression may be restricted to melanoma and or melanocytes as Northern blot analysis indicated that PEPP1 was expressed at very high levels in a melanoma cell line, but not in 7 other non-melanoma cancer cell lines or 12 tissues

that were investigated (Fig 6B). Further work is required to determine whether PEPP1 expression is elevated in all melanoma cells compared to normal melanocytes. It is interesting that a closely related homologue of PEPP1, termed PEPP2, appears to be more widely expressed (Fig 8B). PEPP2 and PEPP3 possess a very similar sequence surrounding the PPBM of their PH domains indicating that they may also interact with PtdIns3P.

Plant cells contain high levels of PtdIns3P as well as PtdIns(3,4)P_2 but no PtdIns(3,4,5)P_3 has been detected [33], consistent with the apparent lack of Class 1A PI 3-kinases in plants. AtPH1 is the first plant protein that has been shown to interact with PtdIns3P and may play an important role as an adaptor protein in regulating signalling processes in plants that are mediated by PtdIns3P. There are no apparent homologues of PEPP1 or AtPH1 in *Drosophila, C.elegans* or *S. cerevisiae.*

The ARF family of GTP binding proteins regulate membrane trafficking and the actin cytoskeleton [34]. A family of ARF GAP proteins, collectively termed centaurins, have been identified and all possess one or more PH domains and an ARF GAP catalytic domain [35]. The PH domain on centaurin-α1 interacts with PtdIns(3,4,5)P_3 and centaurin-α1 is recruited to cell membranes after PI 3-kinase is activated [7]. Recently centaurin-β4 has been shown to be activated by the interaction of its PH domain with PtdIns(4,5)P_2 and, in contrast to centaurin-α1, does not bind to PtdIns(3,4,5)P_3 [36]. The finding in this paper that the uncharacterised ARF GAP protein named centaurin-β2 interacts with PtdIns(3,5)P_2, albeit with moderate affinity, suggests that centaurin-β2 may be regulated by this lipid.

Further investigation is required to establish whether PtdIns(3,5)P_2 can lead to the activation of centaurin-β2. No protein has previously been shown to interact specifically with PtdIns(3,5)P_2 and the physiological processes regulated by this lipid are not known. In yeast, PtdIns(3,5)P_2 is generated in response to osmotic stress [37] by phosphorylation of PtdIns3P at the D5 position by a kinase termed Fab1[38, 39]. There are putative homologues of centaurin-β2 in *Drosophila* (accession number 7595986) and *C.elegans* (accession number 4225944) which possess about 30% overall identity to human centaurin-β2.

In summary, this Example describes a group of novel PH domain containing proteins that possess interesting phosphoinositide binding specificities. TAPP1, TAPP2, FAPP1 and AtPH1 may function as adaptor molecules as they possess no obvious catalytic moieties. In order to further define the physiological processes that are regulated by the PH domain-containing proteins described in this paper it may not only be important to knock out these proteins in cells and mice but also to identify the proteins that they interact with.

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Example 2: Identification of interacting polypeptides

Polypeptides interacting with TAPP1, TAPP2, PEPP1, PEPP2, PEPP3 or FAPP (for example FAPP1 or FAPP2) are identified using yeast two hybrid methods and/or immunoprecipitation/coprecipitation methods. The methods are performed on stimulated and unstimulated cells; polypeptides that interact with TAPP1, TAPP2, PEPP1, PEPP2, PEPP3 or FAPP (for example FAPP1 or FAPP2) in one cell state only (or to different extents in the different cell states) are of particular interest. The methods may also be performed (for comparison) with mutated TAPP1, TAPP2, PEPP1, PEPP2, PEPP3 or FAPP polypeptides, for example mutants which do not bind the relevant phosphoinositide. Coprecipitated polypeptides are analysed by microsequencing and mass spectrometry. The amino acid sequence information is used to identify/isolate polynucleotides encoding the amino acid sequence, using standard molecular biology techniques.

Example 3: Phosphoinositide detection and enzyme assays

Particular enzymes, such as particular lipid phosphatases or inositol lipid kinases, may be assayed using the PH domains described herein, for example using TAPP1, TAPP2, PEPP1, PEPP2, PEPP3 or FAPP (for example FAPP1 or FAPP2). The assay system makes use of the ability of the PH domains to bind specifically to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, when the phosphoinositide is the product (or substrate) of a lipid kinase or phosphatase reaction. The PH domain may be used as a recombinant protein fused to a reporter tag such as a green fluorescent protein or labelled with a fluorescent chromophore.

For example, a Class II PI3 kinase may generate PtdIns3P, which may be measured using PEPP or AtPH1. A PI4 kinase generates PtdIns4P, which may be measured using FAPP. Fab1p[38, 39] generate Ptd(3,5)P_2, which may be measured using centaurin-β2. Alternatively, changes in the substrate for an enzyme may be measured. For example, Fab1p converts PtdIns3P to PtdIns(3,5)P_2 and a PH domain which binds to PtdIns3P (for example the PH domain of PEPP1 or AtPH1) may be used to monitor the level of PtdIns3P and thereby Fab1p activity.

The group of 5' phosphatases target PtdIns(3,4,5)P_3 and also PtdIns4,5P_2, to yield PtdIns4P. Thus, FAPP may be used in measuring such 5' phosphatase activity. FAPP may also be useful in monitoring a 4' phosphatase, for example Sac1p from yeast and homologues thereof, which appears to be specific for dephosphorylating PtdIns4P to phosphoinositide (see, for example, Hughes *et al* (2000) *Bichem J* **350(2)**, 337-352; Nemoto *et al* (2000) *J Biol Chem* **275(44)**, 34293-24305 (rat homologue); Hughes *et al* (2000) *J Biol Chem* **275(2)**, 801-808).

A FRET (fluorescence resonance energy transfer) system may be used. A soliud phase assay with the substrate lipid bound to the surface of a microtitre plate may be used. PH domain binding to the product formed in the immobilised lipid layer is detected by time resolved FRET.

For example, substrate lipids in a lipid layer incorporating a donor chromophore immobilised in wells of a 96 well microtitre plate are incubated with the appropriate enzyme (or sample to be tested for the appropriate enzyme) in the presence of the appropriate recombinant PH domain fused to green fluorescent protein (GFP; including mutant GFPs, as discussed above) and ATP. The PH-GFP binds specifically to the product

(or in an alternative, the substrate) and in doing so is brought into close enough proximity with the chromophore in the lipid layer for FRET to occur. This may be detected using methods well known to those skilled in the art.

This system does not use radioisotopes; does not require separation of reaction products, allowing the system to be used in high throughput screens; does not use lipid vesicles, thereby reducing "false positives" in inhibitor screens due to vesicle disruption by the test compound; and may be used for several enzymes, depending on the lipid and PH domains chosen.

The system may be used for making real time measurements throughout the course of the reaction. Other methods (for example using radioisotopes) may be suitable only for taking measurements at predetermined time points. This may make the present assay system more informative and easier to operate, for example because changes in the activity of the enzyme preparation can be more easily compensated for, for example by making measurements over a shorter or longer period depending on the level of activity of the enzyme, as well known to the skilled person.

In alternative arrangements, the PH domain may be "tagged" in other ways, for example with an alternative chromophore, an epitope tag or a detectable enzyme, as well known in interaction assays, for example immunoassays.

For example, the PH domain may be in the form of a GST fusion protein labelled with a terbium chelate (Terbium Lance Chelate, LKB Wallac) as energy donor and rhodamine labeleld phosphatidylethanolamine as energy acceptor.

It may not be necessary to tag the PH domain. The intrinsic fluorescence of tryptophan residues in the PH domain may change on binding to the phosphoinositide, and this may be used in monitoring the binding of the PH domain to the phosphoinositide, and thereby determining the amount of phosphoinositide present.

The assay configuration may consist of a microtitre plate coated with a mixture containing the substrate phosphoinositide, for example 0.8nmols, phosphatidylserine, 0.7nmols, and rhodamine labelled phosphatidylethanolamine, 0l.5nmmols, giving a total of 2nmols lipid per well. The PH-GST terbium chelate is used at a concentration of 0.175µg/ml in a final volume of 50µl. In order to test the system, a well may be "spiked" with the product lipid at various concentrations. The labelled PH domain is added to the plate and time resolved measurements of fluorescence are taken. For example, excitation at 340nm, emission at 601 nm and a time gate of 50 to 800µsec may be used. Detection limits are in the low pmol range.

Enzyme activity can be determined by measuring fluroescence over time. The enzyme or sample is added with ATP (for example 0.1mM ATP). Data points may be the mean of measurements of several wells (for example eight) read at 30 second intervals over 30 minutes.

In a further alternative, the assay may be run as a homogenous fluid phase assay with the substrate lipid either in free solution or as lipid vesicles. The fluid phase assay relies on reaction product competing for binding in a pre-formed detection complex. The complex may be formed, for example, between Europium lance chelate labelled GST-PH domain, biotinylated

short chain phosphoinositide (for example C6 product phosphoinositide) and streptavidin labelled allophycocyanin (APC). Enzyme activity is detected by the conversion of nonbiotinylated short chain substrate phosphoinositide to product phosphoinositide, which competes for binding with the GST-PH domain in the preformed complex, resulting in a decrease in the FRET signal. The system may be tested by adding biotinylated synthetic short chain product to the assay system. The assay may contain 1µl APC (for example 0.01 to 100 µg, preferably 0.1 to 10 µg), 1µl of the Europium labelled GST-PH domain (for example 0.01 to 100 µg, preferably 0.1 to 10 µg) and increasing concentrations (for example from 0 to 300 pmol) of the water soluble biotinylated short chain product phosphoinositide in a final volume of 50µl. An excitation wavelength of 340, emission wavelength of 665nm and cut-off of 630 nm may be used.

In the assay, non-biotinylated product phosphoinositide produced from the substrate phosphoinositide competes for binding to the GST-PH domain, reducing the observed signal. The system may be tested by addition of increasing amounts of non-biotinylated product phosphoinositide. The biotinylated product phosphoinositide may be present at 0.5µM (25pmol/assay).

A typical assay set-up may be as follows:

Buffer: 50mM HEPES pH7.4, 5mM DTT, 3.5mM $MgCl_2$, 0.02% CHAPS and 250µM ATP.

Detector mix: Eu chelate GST-PH domain (for example 0.01 to 100 µg, preferably 0.1 to 10 µg), streptavidin APC (for example 0.01 to 100 µg, preferably 0.1 to 10 µg), and biotinylated product phosphoinositide 0.5µM.

Enzyme: recombinant enzyme, for example at about 10 ng to 10 µg/ml.

The fluorimeter settings may be excitation 340nm, emission 665 nm, filter 630 nm, time gate 50 to 1050μsec.

The water soluble substrate phosphoinositide may be used at a concentration of 25 μM. The final assay volume may be 50μl.

The rate of decrease of time resolved FRET may be measured over 30 minutes at 30 sec intervals over a range of substrate phosphoinositide concentrations (for example 0 to 70μM) and the initial rates estimated.

As an alternative, the interaction of the components of an assay may be detected using the Alpha Screen™ bead system from BioSignal Packard (part of Packard Biscience), of 1744 rue William, Suite 600, Montreal, Quebec, Canada, H3J 1R4.

CLAIMS

1. The use of a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, in a screening method for identifying a compound suitable for modulating signalling by PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2.

2. The use of claim 1 wherein the polypeptide comprises a PH domain, wherein the PH domain is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3.

3. The use of claim 1 or 2 wherein the polypeptide binds specifically to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2.

4. The use of claim 3 wherein the polypeptide binds specifically to PtdIns(3,4)P_2 and is a polypeptide as defined in claim 27 (TAPP) or a fragment, variant, derivative or fusion thereof, or a fusion of a said fragment, variant or derivative.

5. The use of claim 3 wherein the polypeptide binds specifically to PtdIns4P and is a polypeptide as defined in claim 28 (FAPP) or a fragment, variant, derivative or fusion thereof, or a fusion of a said fragment, variant or derivative.

6. The use of claim 3 wherein the polypeptide binds specifically to PtdIns3P and is a polypeptide as defined in claim 29 (PEPP) or AtPH1 or a fragment, variant, derivative or fusion either thereof, or a fusion of a said fragment, variant or derivative.

7. The use of claim 3 wherein the polypeptide binds specifically to PtdIns(3,5)P_2 and is centaurin-β2 or a fragment, variant, derivative or fusion thereof, or a fusion of a said fragment, variant or derivative.

8. The use according to any of the previous claims wherein the method comprises the steps of (1) exposing the said polypeptide to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2, in the presence of a test compound; (2) determining whether the test compound modulates binding of the said phosphoinositide to the said polypeptide; and (3) selecting a compound which modulates binding of the said phosphoinositide to the said polypeptide.

9. A method of identifying a compound that modulates the phospholipid binding activity of a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, the method comprising contacting a compound with the said polypeptide or a suitable variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative thereof and determining whether the phospholipid binding activity of the said polypeptide or said variant, fragment, derivative or fusion thereof or a fusion of a variant, fragment or derivative thereof is changed in the presence of the compound from that in the absence of said compound.

10. A method of identifying a compound capable of disrupting or preventing the interaction between a polypeptide that is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, and a polypeptide that is capable of binding to the said phosphoinositde-binding polypeptide (interacting polypeptide) wherein the said phosphoinositide-binding polypeptide or a suitable variant,

fragment, derivative or fusion or a fusion of a variant, fragment or derivative thereof, and/or the interacting polypeptide are exposed to the said compound and the interaction between the phosphoinositide-binding polypeptide or variant, fragment, derivative or fusion and the interacting polypeptide in the presence and absence of the compound is measured.

11. A method of identifying a compound that is capable of binding to a polypeptide that is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3 (interacting polypeptide), wherein the said polypeptide or suitable fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative is exposed to the compound and any binding of the compound to the said polypeptide or fragment, variant, derivative or fusion thereof, or fusion of a variant, fragment or derivative is detected and/or measured.

12. A method of identifying a polypeptide (interacting polypeptide) that interacts with a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, the method comprising 1) contacting a) the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative with b) a composition that may contain such an interacting polypeptide, 2) detecting the presence of a complex containing the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative and an interacting polypeptide, and optionally 3) identifying any interacting polypeptide bound to the said phosphoinositide-binding polypeptide or a suitable fragment, variant, derivative or fusion thereof or a suitable fusion of a fragment, variant or derivative.

13. The method of any one of claims 9 to 12 wherein the polypeptide is as defined in any one of claims 2 to 7.

14. The method according to 9 or 10 in which the said binding activity or interaction is decreased.

15. The method according to 9 or 10 in which the said binding activity or interaction is increased.

16. The method of claim 9 to 15 wherein the said method is performed in a cell.

17. A substantially pure interacting polypeptide identified or identifiable by the method according to claim 12.

18. A recombinant polynucleotide encoding or suitable for expressing the interacting polypeptide according to claim 17, or a nucleic acid complementary to the said nucleic acid.

19. A compound identified by or identifiable by the method of any one of claims 9 to 11, 13 to 16.

20. A method of disrupting or preventing the interaction between a polypeptide as defined in any one of claims 1 to 7 (phosphoinositide-binding polypeptide) or a variant, fragment, derivative or fusion, or a fusion of a variant, fragment or derivative, and an interacting polypeptide, as defined in claim 10 or 17 wherein the said interacting polypeptide or phosphoinositide-binding polypeptide or a variant, fragment, derivative or

fusion, or a fusion of a variant, fragment or derivative is exposed to a compound according to claim 19.

21. A method of detecting and/or quantifying PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 in a sample wherein the sample is exposed to a polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3 and the binding of the said polypeptide to any said phosphoinositide present is detected.

22. A method according to claim 21 wherein the polypeptide is as defined in any one of claims 2 to 7

23. A substantially pure polypeptide capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but not capable of binding to PtdIns(3,4,5)P_3, wherein the polypeptide is not full length centaurin-β2 or full length AtPH1[19].

24. The polypeptide of claim 23 wherein the polypeptide comprises a PH domain, wherein the PH domain is capable of binding to PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2 but is not capable of binding to PtdIns(3,4,5)P_3.

25. The polypeptide of claim 23 or 24 wherein the polypeptide binds specifically to one of PtdIns(3,4)P_2, PtdIns3P, PtdIns4P or PtdIns(3,5)P_2.

26. The polypeptide of claim 24 or 25 wherein the PH domain has at least five of the six residues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM).

27. A substantially pure polypeptide comprising the amino acid sequence

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQNLP
SGSSRVGAIKLTYISKVSDATKLRPKAEFCFVMNAGMRKYFLQANDQQDL
VEWVNVLNKAIKITVPKQSDSQPNSDNLSRHGECGKKQVSYRTDIVGGVP
IITPTQKEEVNECGESIDRNNLKRSQSHLPYFTPKPPQDSAVIKAGYCVK
QGAVMKNWKRRYFQLDENTIGYFKSELEKEPLRVIPLKEVHKVQECKQSD
IMMRDNLFEIVTTSRTFYVQADSPEEMHSWIKAVSGAIVAQRGPGRSASS
EHPPGPSESKHAFRPTNAAAATSHSTASRSNSLVSTFTMEKRGFYESLAK
VKPGNFKVQTVSPREPASKVTEQALLRPQSKNGPQEKDCDLVDLDDASLP
VSDV

(human TAPP1 amino acid sequence)

or

RGEREARRVWQADPEIPGARRTRRPEGRPRPM*RAPPEPRPLHGGG*CEQ
SPGMPYVDRQNRICGFLDIEEHENSGKFLRRYFILDTQANCLLWYMDNPQ
NLAMGAGAVGALQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQAND
QKDMKDWVEALNQASKITVPKGGGLPMTTEVLKSLAAPPALEKKPQVAYK
TEIIGGVVVHTPISQNGGDGQEGSEPGSHTILRRSQSYIPTSGCRASTGP
PLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIFFKDV
LKTHECLVKSGDLLMRDNLFEIITSSRTFYVQADSPEDMHSWIKEIGAAV
QALKCHP

(partial human TAPP2 amino acid sequence)

or

MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQnnn
nnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn
nMNAGMRKYFLQANDQQDLVEWVNVLNKAIKITVPKQSDSQPASDSLSRQ
GDCGKKQVSYRTDIVGGVPIITPTQKEEVNECGESLDRNNLKRSQSHLPY
FAPKPPSDSAVIKAGYCVKQGAVMKNWKRRYFQLDENTIGYFKSELEKEP
LRVIPLKEVHKVQECKQSDIMMRDNLFEIVTTSRTFYVQADSPEEMHSWI
KAVSGAIVAQRGPGRSSSSnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnn

(partial mouse TAPP1 amino acid sequence; the run of n's indicates a gap of unknown length)

or

MPYVDRQNRICGFLDIEDNENSGKFLRRYFILDTQANCLLWYMDNPQNLA
VGAGAVGSLQLTYISKVSIATPKQKPKTPFCFVINALSQRYFLQANDQKD
LKDWVEALNQASKITVPKAGTVPLATEVLKNLTAPPTLEKKPQVAYKTEI
IGGVVVQTPISQNGGDGQEGCEPGTHAFLRRSQSYIPTSGCRPSTGPPLI
KSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLRTIPLKDVLKT
HECLVKSGDLLMRDNLFEIITTSRTFYVQADSPEDMHSWIEGIGAAVQAL
KCHPREPSFSRSISLTRPGSSTLTSAPNSILSRRRPPAEEKRGLCKAPSV
ASSWQPWTPVPQAEEKPLSVEHAPEDSLFMPNPGESTATGVLASSRVRHR
SEPQHPKEKPFVFNLDDENIRTSDV

(mouse TAPP2 amino acid sequence)

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

28. A substantially pure polypeptide comprising the amino acid sequence

MEGSRPRSSLSLASSASTISSLSSLSPKKPTRAVNKIHAFGKRGNALRRD
PNLPVHIRGWLHKQDSSGLRLWKRRWFVLSGHCLFYYKDSREESVLGSVL
LPSYNIRPDGPGAPRGRRFTFTAEHPGMRTYVLAADTLEDLRGWLRALGR
ASRAEGDDYGQPRSPARPQPGEGPGGPGGPPEVSRGEEGRISESPEVTRL
SRGRGRPRLLTPSPTTDLHSGLQMRRARSPDLFTPLSRPPSPLSLPRPRS
APARRPPAPSGDT

(partial human PEPP1 amino acid sequence)

or

MEGSRPRSSLSLASSASTISSLSSLSPKKPTRAVNKIHAFGKRGNALRRDP
NLPVHIRGWLHKQDSSGLRLWKRRWFVLSGHCLFYYKDSREESVLGSVLLP
SYNIRPDGPGAPRGRRFTFTAEHPGMRTYVLAADTLEDLRGWLRALGRASR
AEGDDYGQPRSPARPQPGEGPGGPGGPPEVSRGEEGRISESPEVTRLSRGR
GRPRLLTPSPTTDLHSGLQMRRARSPDLFTPLSRPPSPLSLPRPRSAPARR
PPAPSGDTAPPARPHTPLSRIDVRPPLDWGPQRQTLSRPPTPRRGPPSEAG
GGKPPRSPQHWSQEPRTQAHSGSPTYLQLPPRPPGTRASMVLLPGPPLEST
FHQSLETDTLLTKLCGQDRLLRRLQEEIDQKQEEKEQLEAALELTRQQLGQ
ATREAGAPGRAWGRQRLLQDRLVSVRATLCHLTQERERVWDTYSGLEQELG
TLRETLEYLLHLGSPQDRVSAQQQLWMVEDTLAGLGGPQKPPPHTEPDSPS
PVLQGEESSERESLPESLELSSPRSPETDWGRPPGGDKDLASPHLGLGSPR
VSRASSPEGRHLPSPQLGTKAPVARPRMNAQEQLERMRRNQECGRPFPRPT
SPRLLTLGRTLSPARRQPDVEQRPVVGHSGAQKWLRSSGSWSSPRNTTPYL
PTSEGHRERVLSLSQALATEASQWHRMMTGGNLDSQGDPLPGVPLPPSDPT

RQETPPPRSPPVANSGSTGFSRRGSGRGGGPTPWGPAWDAGIAPPVLPQDE
GAWPLRVTLLQSSL

(human PEPP1 amino acid sequence)

or

CKHPVTGQPSQDNCIFVVNEQTVATMTSEEKKERPISMINEASNYNVTSD
YAVHPMSPVGRTSRASKKVHNFGKRSNSIKRNPNAPVVRRGWLYKQDSTG
MKLWKKRWFVLSDLCLFYYRDEKEEGILGSILLPSFQIALLTSEDHINRK
YAFKAAHPNMRTYYFCTDTGKEMELWMKAMLDAALVQTEPVKRVDKITSE
NAPTKETNNIPNHRVLIKPEIQNNQKNKEMSKIEEKKALEAEKYGFQKDG
QDRPLTKINSVKLNSLPSEYESGSACPAQTVHYRPINLSSSENKIVNVSL
ADLRGGNRPNTGPLYTEADRVIQRTNSMQQLEQWIKIQKGRGHEEETRGV
ISYQTLPRNMPSHRAQIMARYPEGYRTLPRNSKTRPESICSVTPSTHDKT
LGPGAEEKRRSMRDDTMWQLYEWQQRQFYNKQSTLPRHSTLSSPKTMVNI
SDQTMHSIPTSPSHGSIAAYQGYSPQRTYRSEVSSPIQRGDVTIDRRHRA
HHPKVK

(partial human PEPP2 amino acid sequence)

or

MAADLNLEWISLPRSWTYGITRGGRVFFINEEAKSTTWLHPVTGEAVVTG
HRRQSTDLPTGWEEAYTFKGARYYINHNERKVTCKHPVTGQPSQDNCIFV
VNEQTVATMTSEEKKERPISMINEASNYNVTSDYAVHPMSPVGRTSRASK
KVHNFGKRSNSIKRNPNAPVVRRGWLYKQDSTGMKLWKKRWFVLSDLCLF
YYRDEKEEGILGSILLPSFQIALLTSEDHINRKYAFKAAHPNMRTYYFCT
DTGKEMELWMKAMLDAALVQTEPVKRVDKITSENAPTKETNNIPNHRVLI
KPEIQNNQKNKEMSKIEEKKALEAEKYGFQKDGQDRPLTKINSVKLNSLP
SEYESGSACPAQTVHYRPINLSSSENKIVNVSLADLRGGNRPNTGPLYTE
ADRVIQRTNSMQQLEQWIKIQKGRGHEEETRGVISYQTLPRNMPSHRAQI
MARYPEGYRTLPRNSKTRPESICSVTPSTHDKTLGPGAEEKRRSMRDDTM
WQLYEWQQRQFYNKQSTLPRHSTLSSPKTMVNISDQTMHSIPTSPSHGSI
AAYQGYSPQRTYRSEVSSPIQRGDVTIDRRHRAHHPKHVYVPDRRSVPAG
LTLQSVSPQSLQGKTLSQDEGRGTLYKYRPEEVDIDAKLSRLCEQDKVVH
ALEEKLQQLHKEKYTLEQALLSASQEIEMHADNPAAIQTVVLQRDDLQNG
LLSTCRELSRATAELERAWREYDKLEYDVTVTRNQMQEQLDHLGEVQTES
AGIQRAQIQKELWRIQDVMEGLSKHKQQRGTTEIGMIGSKPFSTVKYKNE
GPDYRLYKSEPELTTVAEVDESNGEEKSEPVSEIETSVVKGSHFPVGVVP
PRAKSPTPESSTIASYVTLRKTKKMMDLRTERPRSAVEQLCLAESTRPRM
TVEEQMERIRRHQQACLREKKKGLNVIGASDQSPLQSPSNLRDNPFRTTQ
TRRRDDKELDTAIRENDVKPDHETPATEIVQLKETEPQNVDFSKELKKTE

NISYEMLFEPEPNGVNSVEMMDKERNKDKMPEDVTFSPQDETQTANHKPE
EHPEENTKNSVDEQEETVISYESTPEVSRGNQTMAVKSLSPSPESSASPV
PSTQPQLTEGSHFMCV

(human PEPP2)

or

MSNKTGGKRPATTNSDIPNHNMVSEVPPERPSVRATRTARKAIAFGKRSH
SMKRNPNAPVTKAGWLFKQASSGVKQWNKRWFVLVDRCLFYYKDEKEESI
LGSIPLLSFRVAAVQPSDNISRKHTFKAEHAGVRTYFFSAESPEEQEAWI
QAMGEAARVQIPPAQKSVPQAVRHSHEKPDSENVPPSKHHQQPPHNSLPK
PEPEAKTRGEGDGRGCEKAERRPERPEVKKEPPVKANGLPAGPEPASEPG
SPYPEGPRVPGGGEQPAQPNGWQYHSPSRPGSTAFPSQDGETGGHRRSFP
PRTNPDKIAQRKSSMNQLQQWVNLRRGVPPPEDLRSPSRFYPVSRRVPEY
YGPYSSQYPDDYQYYPPGVRPESICSMPAYDRISPPWALEDKRHAFRNGG
GPAYQLREWKEPASYGRQDATVWIPSPSRQPVYYDELDAASSSLRRLSLQ
PRSHSVPRSPSQGSYSRARIYSPVRSPSARFERLPPRSEDIYADPAAYVM
RRSISSPKVPPYPEVFRDSLHTYKLNEQDTDKLLGKLCEQNKVVREQDRL
VQQLRAEKESLESALMGTHQELEMFGSQPAYPEKLRHKKDSLQNQLINIR
VELSQATTALTNSTIEYEHLESEVSALHDDLWEQLNLDTQNEVLNRQIQK
EIWRIQDVMEGLRKNNPSRGTDTAKHRGGLGPSATYSSNSPASPLSSASL
TSPLSPFSLVSGSQGSPTKPGSNEPKANYEQSKKDPHQTLPLDTPRDISL
VPTRQEVEAEKQAALNKVGVVPPRTKSPTDDEVTPSAVVRRNASGLTNGL
SSQERPKSAVFPGEGKVKMSVEEQIDRMRRHQSGSMKEKRRSLQLPASPA
PDPSPRPAYKVVRRHRSIHEVDISNLEAALRAEEPGGHAYETPREEIARL
RKMELEPQHYDVDINKELSTPDKVLIPERYIDLEPDTPLSPEELKEKQKK
VERIKTLIAKSSMQNVVPIGEGDSVDVPQDSESQLQEQEKRIEISCALAT
EASRRGRMLSVQCATPSPPTSPASPAPPANPLSSESPRGADSSYTMRV

(human PEPP3 amino acid sequence)

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

29. A substantially pure polypeptide comprising the amino acid sequence

MEGVLYKWTNYLTGWQPRWFVLDNGILSYYDSQDDVCKGSKGSIKMAVCE
IKVHSADNTRMELIIPGEQHFYMKAVNAAERQRWLVALGSSKACLTDTRT
KKEKEISETSESLKTKMSELRLYCDLLMQQVHTIQEFVHHDENHSSPSAE

NMNEASSLLSATCNTFITTLEECVKIANAKFKPEMFQLHHPDPLVSPVSP
SPVQMMKRSVSHPGSCSSERSSHSIKEPVSTLHRLSQRRRRTYSDTDSCS
DIPLEDPDRPVHCSKNTLNGDLASATIPEESRLTAKKQSESEDTLPSFSS

(human FAPP1 amino acid sequence)

or

MEGVLYKWTNYLTGWQPRWFVLDNGILSYYDSQDDVCKGSKGSIKMAVCE
IKVHSADNTRMELIIPGEQHFYMKAVNAAERQRWLVALGSSKACLTDTRT
KKEKEISETSESLKTKMSELRLYCDLLMQQVHTIQEFVHHDENHSSPSAE
NMNEASSLLSATCNTFITTLEECVKIANAKFKPEMFQLHHPDPLVSPVSP
SPVQMMKRSVSHPGSCSSERSSHSIKEPVSTLHRLSQRRRRTYSDTDSCS
DIPLEDPDRPVHCSKNTLNGDLASATIPEESRLTAKKQSESEDTLPSFSS

(mouse FAPP1 amino acid sequence)

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

30. A substantially pure polypeptide comprising the amino acid sequence

DVRAMLRGSRLRKIRSRTWHKERLYRLQED

or

FEGTLYKRGALLKGWKPRWFVLNVT (PH30)

or

RPGLRALKKMGLTEDEDEDVRAMLRGSRLRKIRSRTWHKERLYRLQEDGL
SVWFQRRIPRAPSQHIFFVQHIEAVREGHQSEGLRRFGGAFAPARCLTIA
FKGRRKNLDLAAPTAEEAQRWVRGLTKLRARLDAMSQRERLDHWIHSYLH
RADSNQDSKMSFKEIKSLLRILV

(PH83)

or

KEGNLKKKGGGEGGRNWTVRWFKLKND

(*Dictyostelium* PH domain polypeptide)

or a variant, fragment, fusion or derivative thereof, or a fusion of a said variant, fragment, fusion or derivative thereof.

31. A polypeptide according to claim 31 wherein the polypeptide comprises a PH domain, preferably a PH domain that has at least five of the six residues of a Putative PtdIns(3,4,5)P_3 Binding Motif (PPBM).

32. A recombinant polynucleotide suitable for expressing a polypeptide according to any one of claims 23 to 31.

33. A vector suitable for replication in a mammalian/eukaryotic cell comprising a polynucleotide encoding the polypeptide, variant, fragment, derivative or fusion according to any one of claims 23 to 31.

34. A polynucleotide or vector according to any one of claims 32 to 34 or 18 which contains no introns.

35. A host cell comprising a recombinant polynucleotide or a replicable vector as defined in any one of Claims 32 to 34 or 18.

36. A method of making a polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative the method comprising culturing a host cell as defined in Claim 35 which expresses said polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative and isolating said polypeptide or a variant, fragment, derivative or fusion thereof or fusion of a said variant, or fragment or derivative.

37. The method of claim 16 wherein the said host cell is a eukaryotic cell.

38. A polypeptide, or a variant, fragment, derivative or fusion thereof or fusion of a said variant or fragment or derivative obtainable by the method of Claim 37.

39. An antibody reactive towards a polypeptide according to any one of claims 23 to 31.

40. An antibody according to Claim 39 wherein the antibody does not react substantially with another polypeptide comprising a PH domain.

41. A polypeptide as defined in any one of claims 23 to 31 or claim 17 or a fragment, fusion, variant or derivative thereof, or fusion of a fragment, variant or derivative, for use in medicine.

42. A nucleic acid encoding, or complementary to a nucleic acid encoding, a polypeptide as defined in claim 41 for use in medicine.

43. A compound as defined in claim 19 or an antibody as defined in claim 39 or 40 for use in medicine.

44. A compound capable of altering the expression of a polypeptide as defined in any one of claims 23 to 31.

45. A compound according to claim 44 for use in medicine.

46. A pharmaceutical composition comprising a polypeptide, interacting polypeptide, nucleic acid, antibody and/or compound as defined in any one of claims 41 to 44 and a pharmaceutically acceptable carrier.

47. A method of treating a patient in need of modulation of the activity of a polypeptide as defined in any one of claims 23 to 31, or with an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition), the method comprising administering to the patient an effective amount of a polypeptide, interacting polypeptide, nucleic acid, antibody and/or compound as defined in any one of claims 41 to 44.

48. Use of a polypeptide, interacting polypeptide, nucleic acid, antibody and/or compound as defined in any one of claims 41 to 44 in the manufacture of a medicament for treatment of a patient in need of modulation of the activity of a polypeptide as defined in any one of claims 23 to 31, or with an inflammatory or an ischaemic disease, cancer (particularly melanoma), diabetes, thrombosis or a defect in glycogen metabolism (or at risk of such a condition).

49. A method of determining the susceptibility of a patient to cancer, for example melanoma, comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer, for example melanoma.

50. A method of diagnosing cancer, for example melanoma, in a patient comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer, for example melanoma.

51. A method of predicting the relative prospects of a particular outcome of a cancer, for example melanoma, in a patient comprising the steps of (i) obtaining a sample containing nucleic acid and/or protein from the patient; and (ii) determining whether the sample contains a level of PEPP nucleic acid or protein associated with cancer.

52. Any novel polypeptide or nucleic acid as herein disclosed.

53. A method according to claim 21 or 22 wherein the method is performed in cells.

54. A method according to claim 21 or 22 wherein the method is performed in the absence of cells.

55. A method according to any one of claims 21, 22, 53 or 54 wherein the said polypeptide comprises a chromophore.

56. A method for detecting or quantifying lipid kinase or phosphatase activity wherein a method according to any one of claims 21, 22, 53, 54 or 55 is used.

57. A method for identifying a modulator of a lipid kinase or phosphatase activity wherein the lipid kinase or phosphatase activity is measured in the presence of the compound using a method according to any one of claims 21, 22, 53, 54 or 55.

58. A kit of parts useful in carrying out a method according to any one of claims 21, 22, 53 to 57.

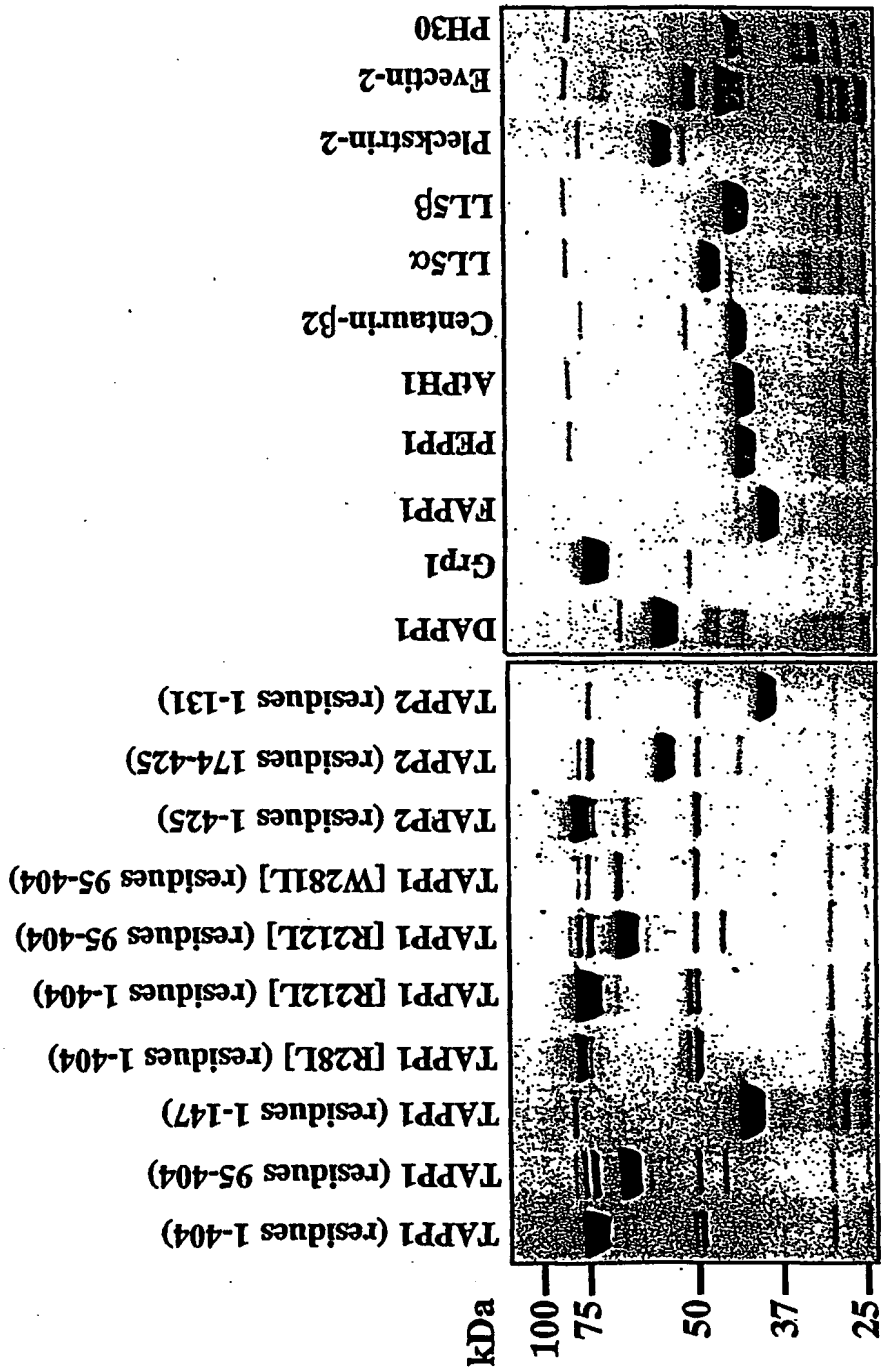
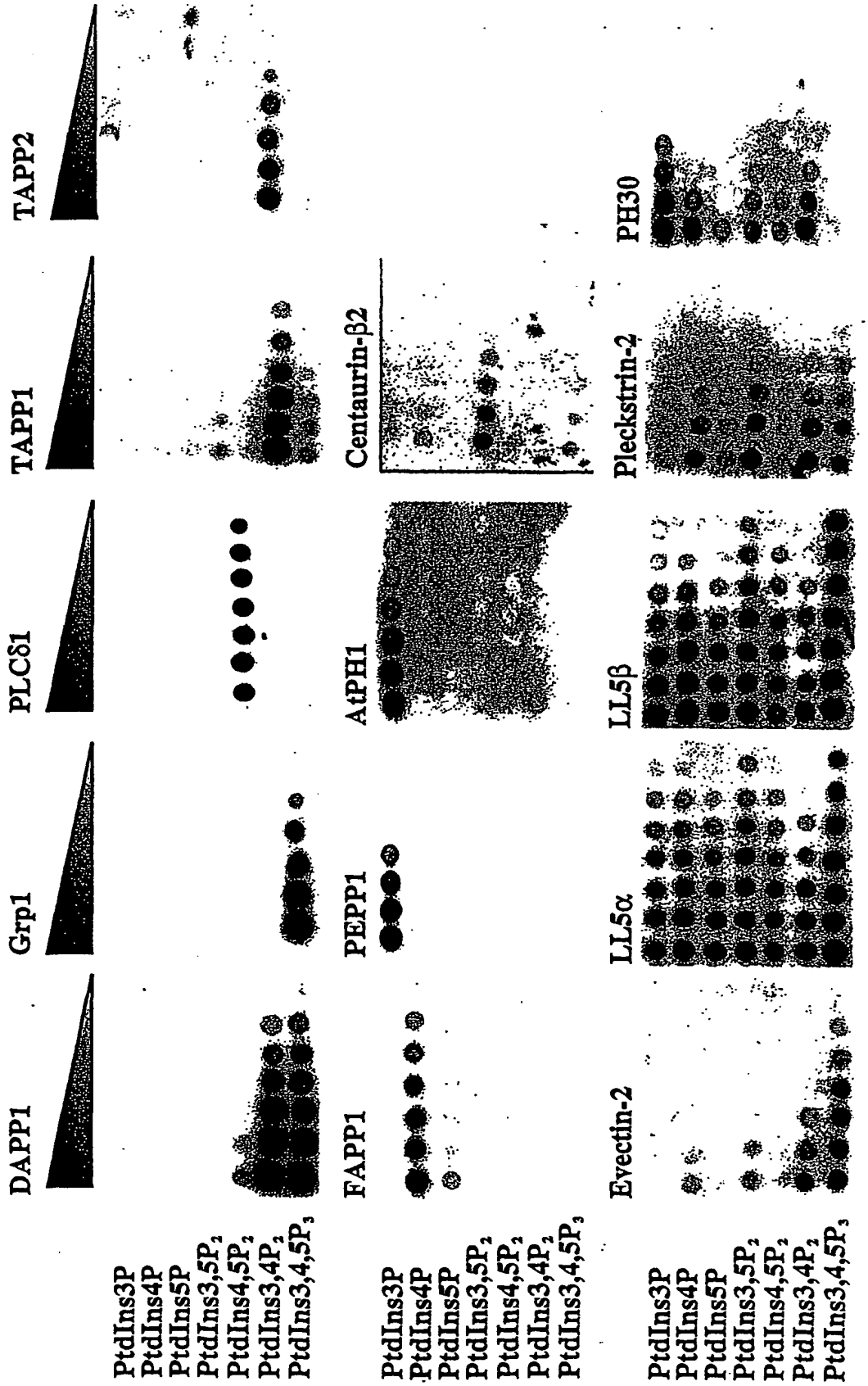

kDa
100
75
50
37
25
TAPP1 (residues 1-404)
TAPP1 (residues 95-404)
TAPP1 (residues 1-147)
TAPP1 [R28L] (residues 1-404)
TAPP1 [R212L] (residues 1-404)
TAPP1 [R212L] (residues 95-404)
TAPP1 [W28IL] (residues 95-404)
TAPP2 (residues 1-425)
TAPP2 (residues 174-425)
TAPP2 (residues 1-131)
DAPP1
Grp1
FAPP1
PEPP1
ARPH1
Centaurin-β2
LL5α
LL5β
Pleckstrin-2
Evectin-2
PH30

Figure 1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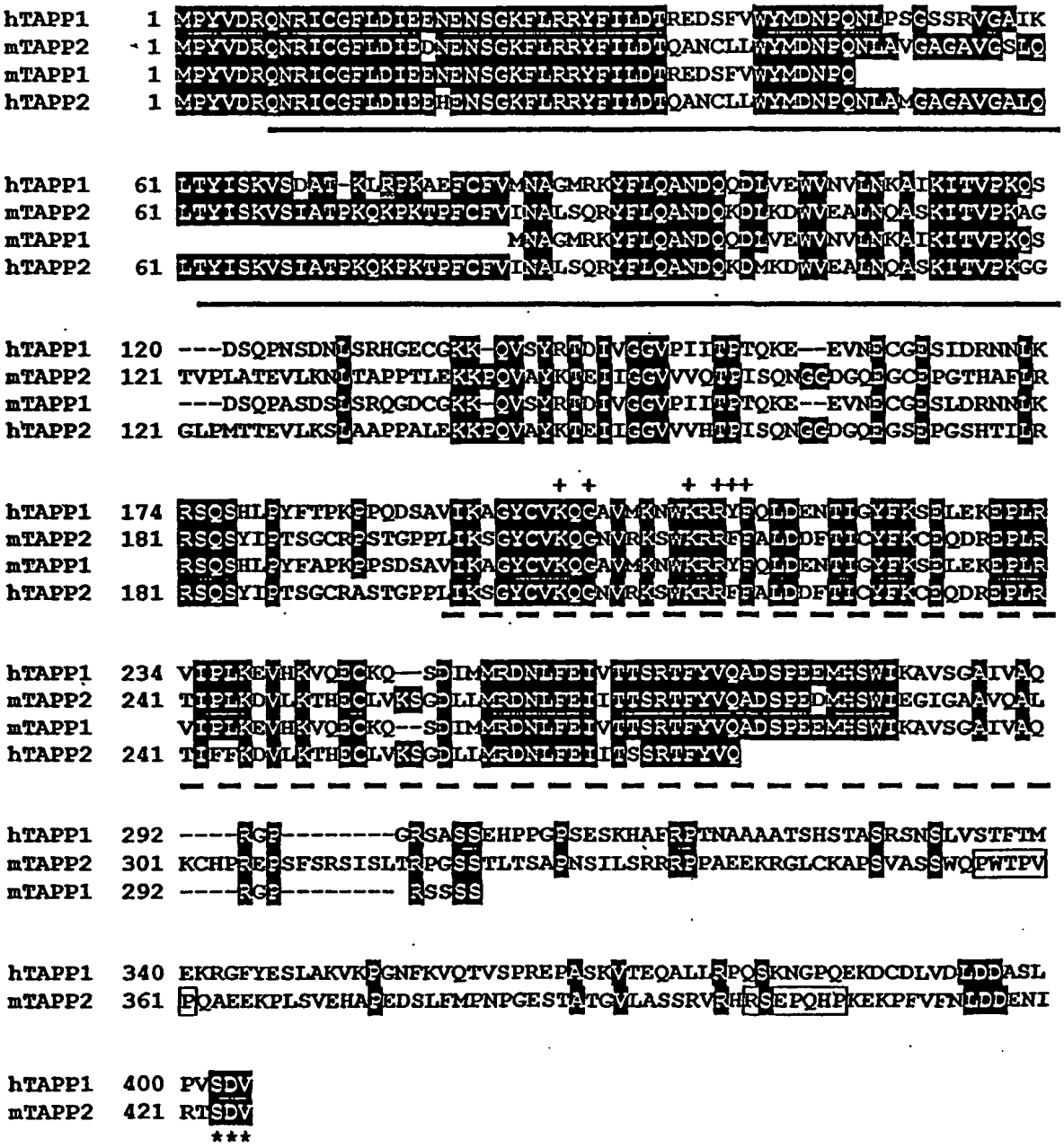
Grp1
PLCδ1
TAPP1
TAPP2
FAPP1
PEPP1
AtPH1
Centaurin-β2
Evectin-2
LL5α
LL5β
Pleckstrin-2
PH30
PtdIns3P
PtdIns4P
PtdIns5P
PtdIns3,5P2
PtdIns4,5P2
PtdIns3,4P2
PtdIns3,4,5P3

Figure 2

Figure 3A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mTAPP1 1 MPYVDRQNRICGFLDIEENENSGKFLRRYFILDTREDSFVWYMDNPQ
hTAPP2 1 MPYVDRQNRICGFLDIEEHENSGKFLRRYFILDTQANCLLWYMDNPQNLAMGAGAVGALQ
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mTAPP2 61 LTYISKVSIATPKQKPKTPFCFVINALSQRYFLQANDQKDLKDWVEALNQASKITVPKAG
mTAPP1 MNAGMRKYFLQANDQQDLVEWVNVLNKAIKITVPKQS
hTAPP2 61 LTYISKVSIATPKQKPKTPFCFVINALSQRYFLQANDQKDMKDWVEALNQASKITVPKGG
hTAPP1 120 ---DSQPNSDNLSRHGECGKK-QVSYRTDIVGGVPIITPTQKE--EVNECGESIDRNNLK
mTAPP2 121 TVPLATEVLKNLTAPPTLEKKPQVAYKTEIIGGVVVQTPISQNGGDGQEGCEPGTHAFLR
mTAPP1 ---DSQPASDSLSRQGDCGKK-QVSYRTDIVGGVPIITPTQKE--EVNECGESLDRNNLK
hTAPP2 121 GLPMTTEVLKSLAAPPALEKKPQVAYKTEIIGGVVVHTPISQNGGDGQEGSEPGSHTILR
hTAPP1 174 RSQSHLPYFTPKPPQDSAVIKAGYCVKQGAVMKNWKRRYFQLDENTIGYFKSELEKEPLR
mTAPP2 181 RSQSYIPTSGCRPSTGPPLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLR
mTAPP1 RSQSHLPYFAPKPPSDSAVIKAGYCVKQGAVMKNWKRRYFQLDENTIGYFKSELEKEPLR
hTAPP2 181 RSQSYIPTSGCRASTGPPLIKSGYCVKQGNVRKSWKRRFFALDDFTICYFKCEQDREPLR
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mTAPP2 241 TIPLKDVLKTHECLVKSGDLIMRDNLFEIITTSRTFYVQADSPEDMHSWIEGIGAAVQAL
mTAPP1 VIPLKEVHKVQECKQ--SDIMMRDNLFEIVTTSRTFYVQADSPEEMHSWIKAVSGAIVAQ
hTAPP2 241 TIFFKDVLKTHECLVKSGDLIMRDNLFEIITSSRTFYVQ
hTAPP1 292 -----RGE--------GRSASSEHPPGPSESKHAFRPTNAAAATSHSTASRSNSLVSTFTM
mTAPP2 301 KCHPREPSFSRSISLTRPGSSTLTSAPNSILSRRRPPAEEKRGLCKAPSVASSWQPWTPV
mTAPP1 292 -----RGE-------- RSSSS
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mTAPP2 361 PQAEEKPLSVEHAPEDSLFMPNPGESTATGVLASSRVRHRSEPQHPKEKPFVFNLDDENI
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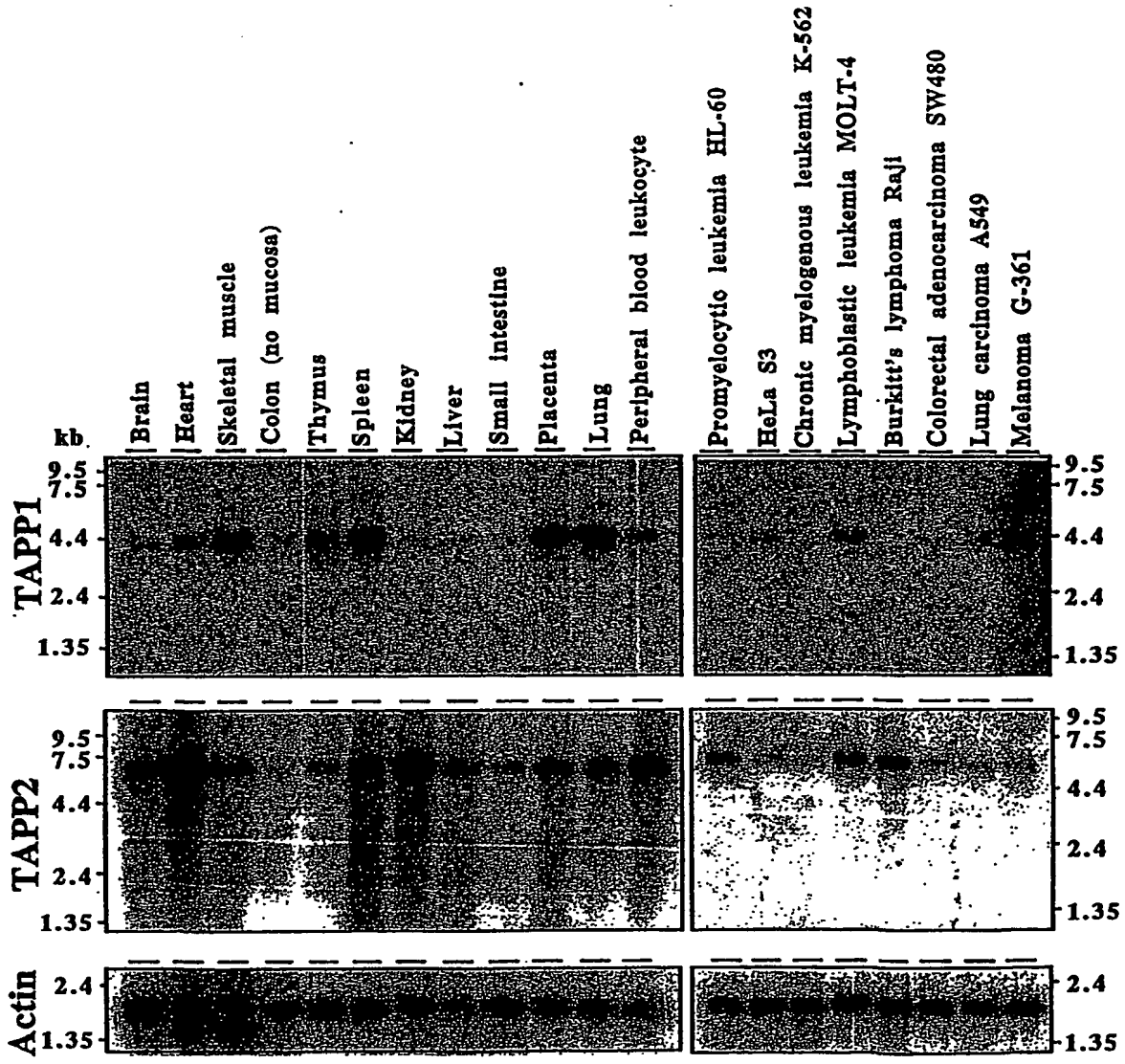
Figure 3B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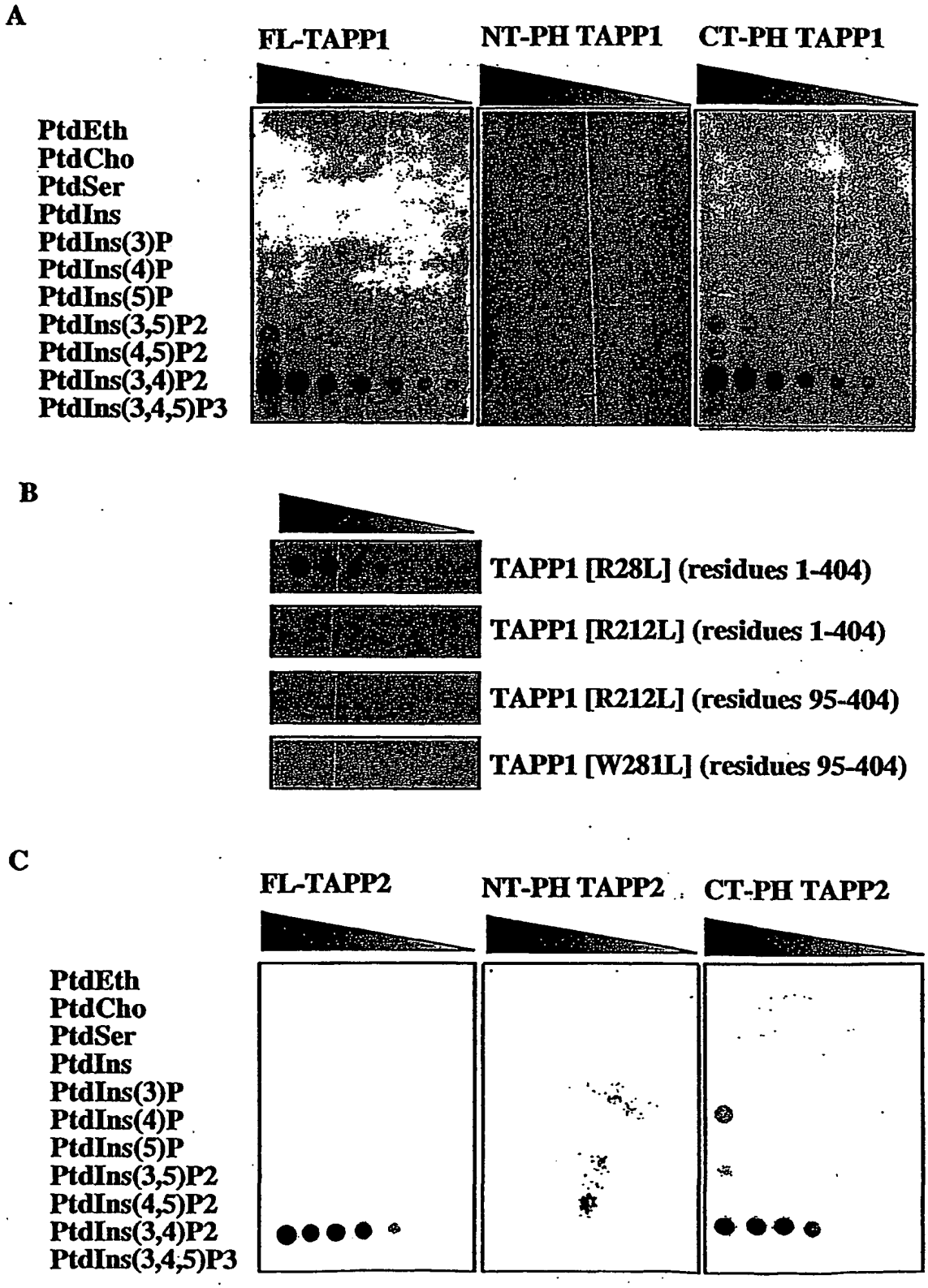
Heart
Skeletal muscle
Colon (no mucosa)
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Peripheral blood leukocyte
Promyelocytic leukemia HL-60
HeLa S3
Chronic myelogenous leukemia K-562
Lymphoblastic leukemia MOLT-4
Burkitt's lymphoma Raji
Colorectal adenocarcinoma SW480
Lung carcinoma A549
Melanoma G-361
TAPP1
TAPP2
Actin
9.5
7.5
4.4
2.4
1.35

Figure 4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CT-PH TAPP1
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PtdCho
PtdSer
PtdIns
PtdIns(3)P
PtdIns(4)P
PtdIns(5)P
PtdIns(3,5)P2
PtdIns(4,5)P2
PtdIns(3,4)P2
PtdIns(3,4,5)P3
B
TAPP1 [R28L] (residues 1-404)
TAPP1 [R212L] (residues 1-404)
TAPP1 [R212L] (residues 95-404)
TAPP1 [W281L] (residues 95-404)
C
FL-TAPP2
NT-PH TAPP2
CT-PH TAPP2
PtdEth
PtdCho
PtdSer
PtdIns
PtdIns(3)P
PtdIns(4)P
PtdIns(5)P
PtdIns(3,5)P2
PtdIns(4,5)P2
PtdIns(3,4)P2
PtdIns(3,4,5)P3

+ + + +++
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Zebrafish 1 MEGVLYKWTNYMTGWQPRWFVLDNGITSYYDSQDDVCKGSKGSIKMPVCEIKVHPTDNTR
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Zebrafish 121 RLYCDLLMQQVHTIQESVEQHE
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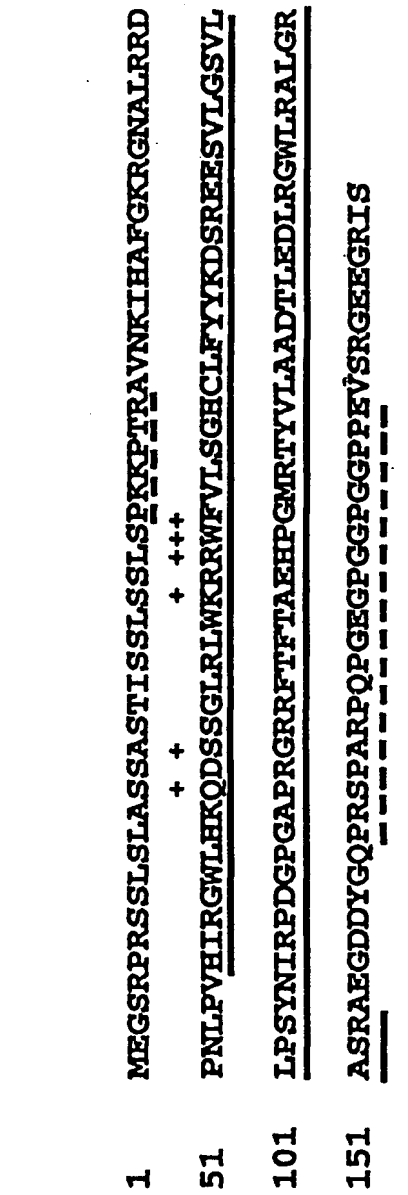
Figure 5

A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+ + + +++
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Figure 6A

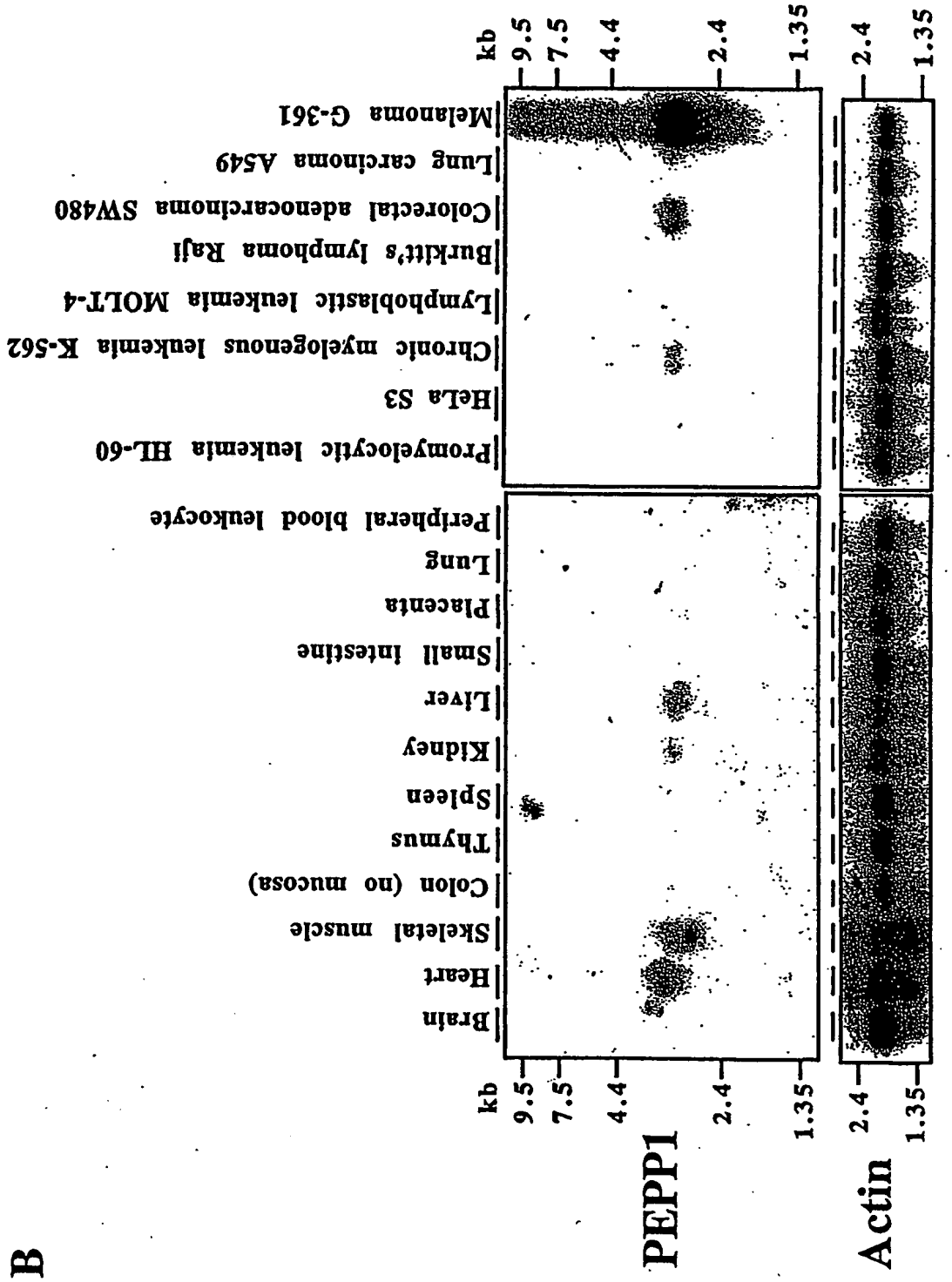

B
PEPP1
Actin
kb
9.5
7.5
4.4
2.4
1.35
Brain
Heart
Skeletal muscle
Colon (no mucosa)
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Peripheral blood leukocyte
Promyelocytic leukemia HL-60
HeLa S3
Chronic myelogenous leukemia K-562
Lymphoblastic leukemia MOLT-4
Burkitt's lymphoma Raji
Colorectal adenocarcinoma SW480
Lung carcinoma A549
Melanoma G-361

Figure 6B

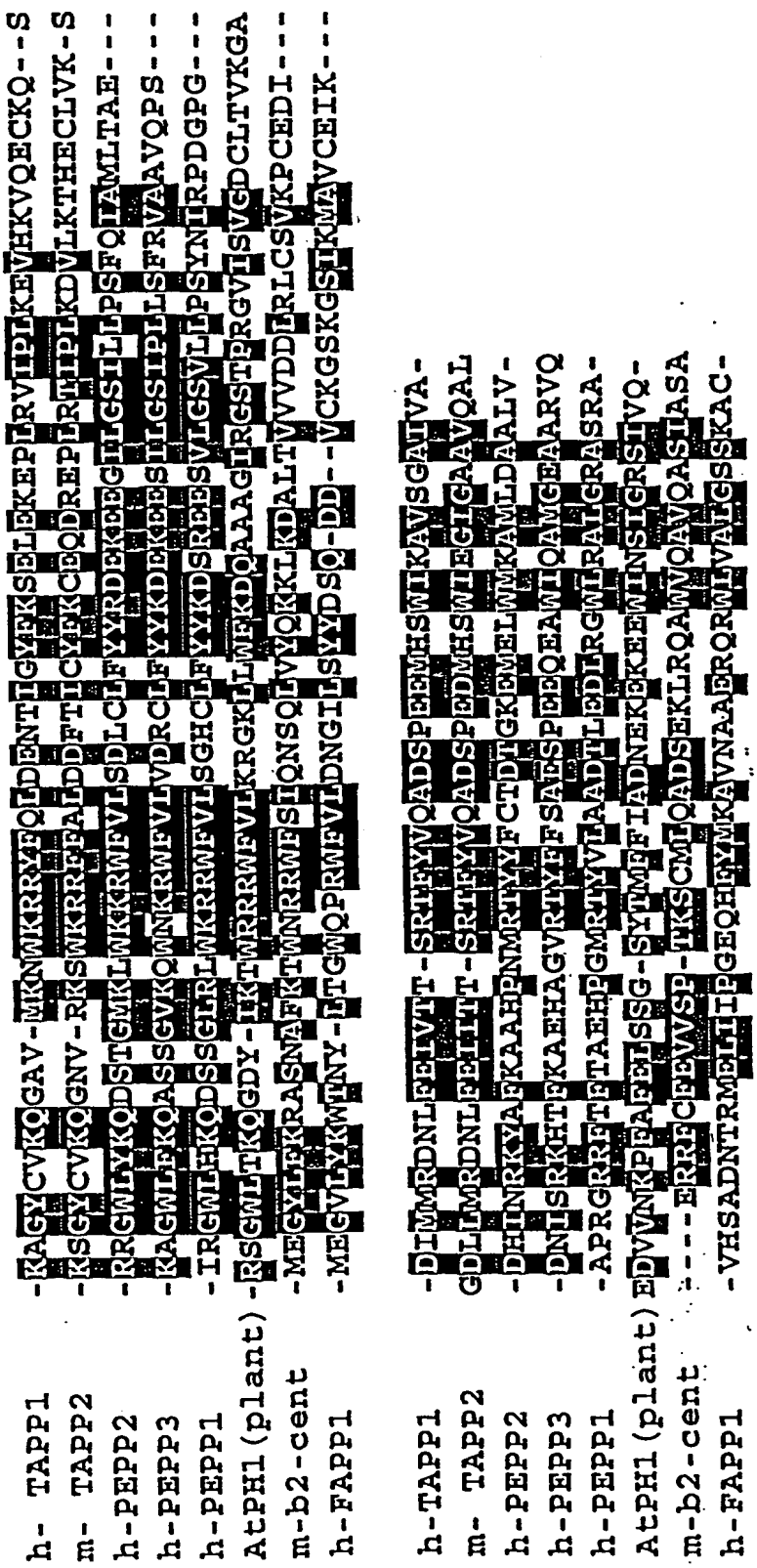
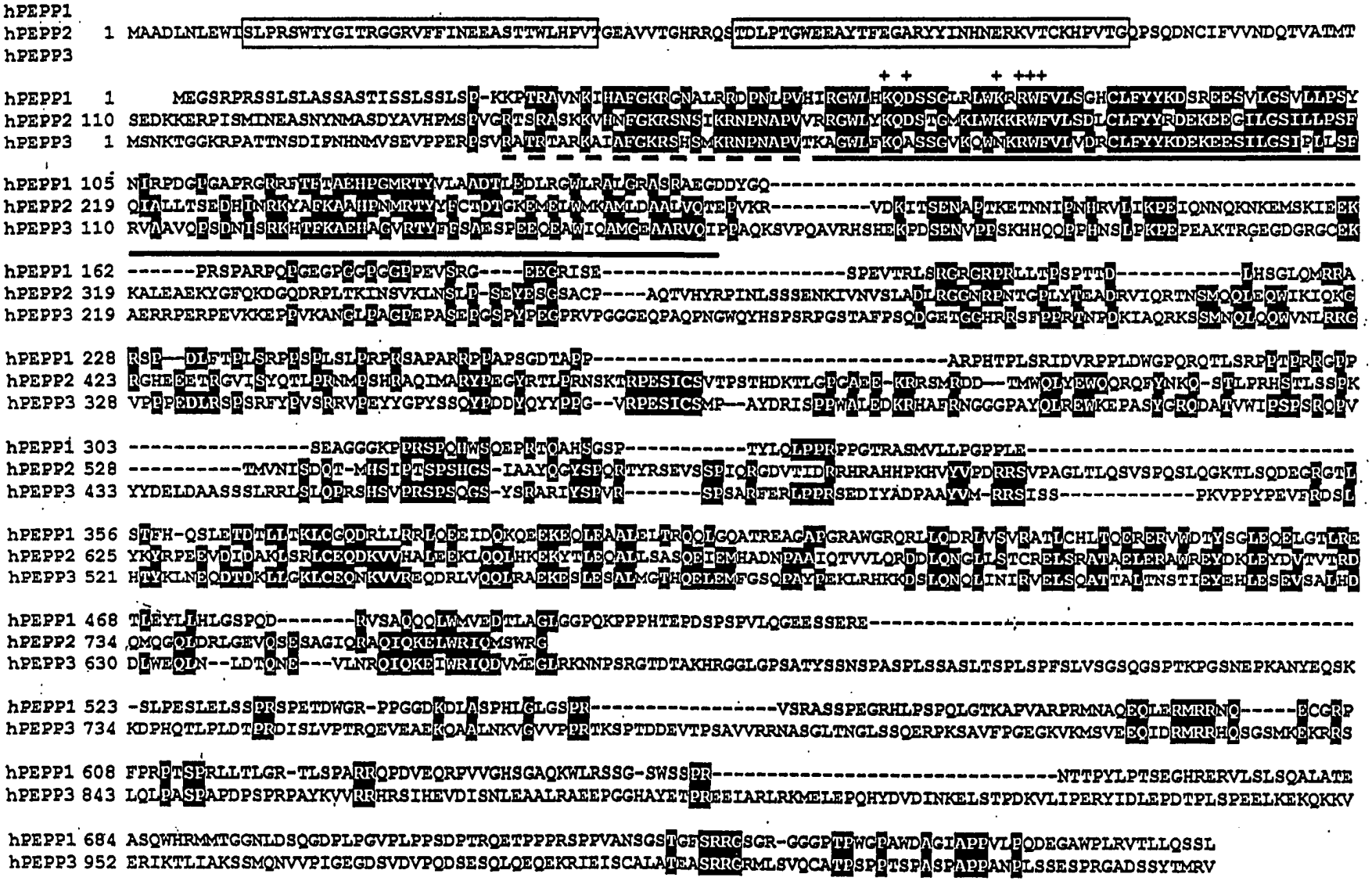


Figure 7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hPEPP1 1 MEGSRPRSSLSLASSASTISSLSSLSP-KKPTRAVNKIHAFGKRGNALRRDPNLPVHIRGWLHKQDSSGLRLWKRRWFVLSGHCLFYYKDSREESVLGSVLLPSY
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hPEPP3 110 RVNAVQPSDNISRKHTFKAEHAGVRTYFFSAESPEEQEAWIQAMGEAARVQIPPAQKSVPQAVRHSHEKPDSENVPPSKHHQQPPHNSLPKPEPEAKTRGEGDGRGCEK
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hPEPP2 319 KALEAEKYGFQKDGQDRPLTKINSVKLNSLP-SEYESGSACP----AQTVHYRPINLSSSENKIVNVSLADLRGCNRPNTGPLYTEADRVIQRTNSMQQLEQWIKIQKG
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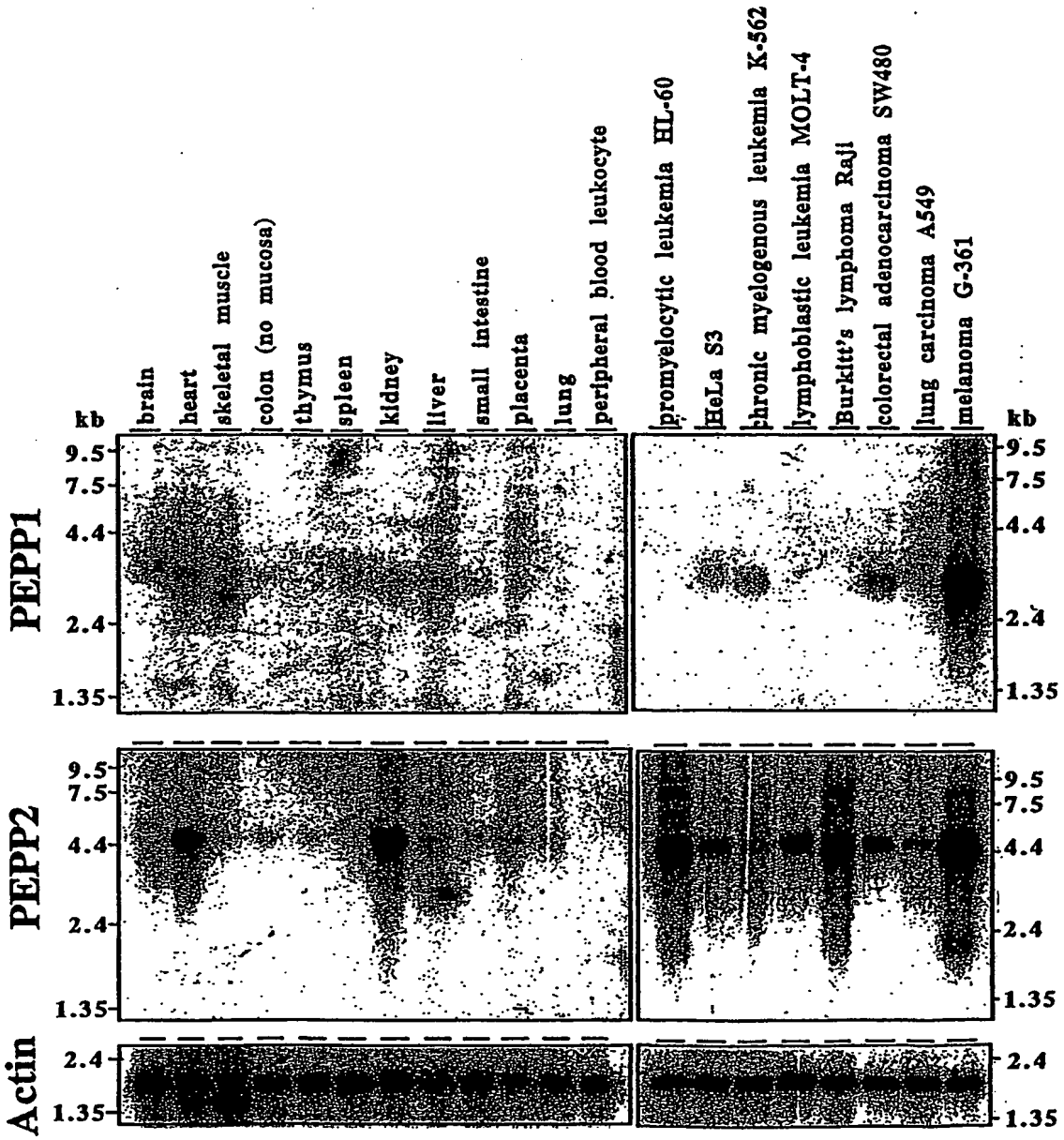
Figure 8A

Figure 8B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heart
skeletal muscle
colon (no mucosa)
thymus
spleen
kidney
liver
small intestine
placenta
lung
peripheral blood leukocyte
promyelocytic leukemia HL-60
HeLa S3
chronic myelogenous leukemia K-562
lymphoblastic leukemia MOLT-4
Burkitt's lymphoma Raji
colorectal adenocarcinoma SW480
lung carcinoma A549
melanoma G-361
kb
9.5
7.5
4.4
2.4
1.35
PEPP1
PEPP2
Actin

Figure 9 (page 2 of 2)

GGTGCTCCTCGCCTCTTGGGGCCTGGGGCAGTGAGGGGGCCGGCGGGCGT
GGGCCGAGTGGCCGCGGGCGCCATGGAGGGGGTGCTGTACAAGTGGACCA
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ATACTTTTCTGAAGACCTTGGAAGAATGCATGCAGATTGCAAATGCAGCC
TTCACCTCTGAGCTGCTCTACCACACTCCACCAGGATCACCACAGCTGGC
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CCTCCCTGCCATGAAGAAGCAGATGGCCATACTGGACGCTTTATAAGAGG
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MEGVLYKWTNYLSGWQPRWFLLCGGILSYYDSPEDAWKGCKGSIQMAVCE
IQVHSVDNTRMDLIIPGEQYFYLKARSVAERQRWLVALGSAKACLTDSRT
QKEKEFAENTENLKTKMSELRLYCDLLVQQVDKTKEVTTTGVSNSEEGID
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WEEGKEVIPTFFSTMNTSFSDIELLEDSGIPTEAFLASCCAVVPVLDKLG
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ATEALLWLKRGLKFLKGFLTEVKNGEKDIQTALNNAYGKTLRQHHGWVVR
GVFALALRATPSYEDFVAALTVKEGDHRKEAFSIGMQRDLSLYLPAMKKQ
MAILDAL*

Figure 9 (page 1 of 2)

```
FAPP1    1 MEGVLYKWTNYLTGWQPRWFMLDNGILSYYDSQDDVCKGSKGSIKMAVCEIKVHSADNTR
FAPP2    1 MEGVLYKWTNYLSGWQPRWFTLCGGILSYYDSPEDAWKGCKGSIQMAVCEIQVHSVDNTR

FAPP1   61 MELITPGEQHFYMKAVNAAERQRWLVALGSSKACLTDTRTKKEKEISETSESLKTKMSEL
FAPP2   61 MDLITIPGEQYFYEKARSVAERQRWLVALGSAKACLTDSRTQKEKEFAENTENLKTKMSEL

FAPP1  121 RLYCDLLMQQVHTIQEFVHHDENHSSPSAENMNEASSLLSATCNTFTTTLEECVKIANAK
FAPP2  121 RLYCDLLMQQVDKTKEVTTTGVSNS----EGIDVGTLLKSTCNTFTKTLEECMQIANAA

FAPP1  181 FKPEMFQLHHP-DPLVSPVSPS----PVQMMKRSVSTPGSCSSERSSHSIKEPVSTLHRL
FAPP2  177 TTSELLYHTPPGSPQLAMLKSSKMKHPHIPIHNSIERQTELSHCENGSLNMEINGEEEIL

FAPP1  236 SQRTR--RTYSDTDSCSDIPLEDPDRPVHCSKNTLNGDLASATIPEESRLTAKKQS----
FAPP2  237 MKNKNSIYLKSAEIDCSISSEENTDDNLTVQGEIMKEDRMENLKNHDNNLSQSGSDSSCS

FAPP1  290 ------ESEDTGPSFSS*
FAPP2  297 PECLWEEGKEVIPHFFSTMNTSFSDIELLEDSGIPTEAFLASCCAVVPVLDKLGPTVFAP

FAPP2  357 VKMDLVENIKKVNQKYITNKEEFTTLQKIVLHEVEADVAQVRNSATEALLWLKRGLKFLK
FAPP2  417 GFLTEVKNGEKDIQTALNNAYGKTLRQHHGWVVRGVFALALRATPSYEDFVAALTVKEGD
FAPP2  477 HRKEAFSIGMQRDLSLYLPAMKQMAILDAL*
```

Figure 10

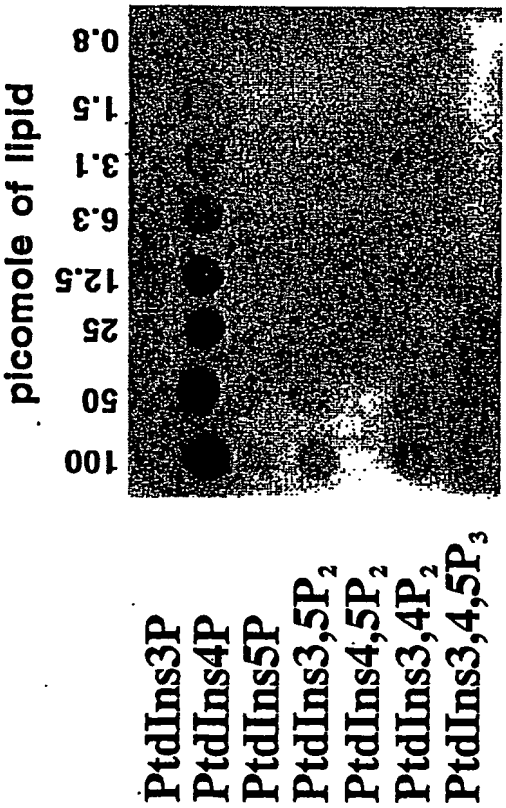
picomole of lipid
100
50
25
12.5
6.3
3.1
1.5
0.8
PtdIns3P
PtdIns4P
PtdIns5P
PtdIns3,5P2
PtdIns4,5P2
PtdIns3,4P2
PtdIns3,4,5P3

Figure 11